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SOIL CONSUMPTION OF ATMOSPHERIC METHANE: IMPORTANCE OF MICROBIAL PHYSIOLOGY AND DIVERSITY

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Jay Gulledge, M.S.

Fairbanks, Alaska

August 1996

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SOIL CONSUMPTION OF ATMOSPHERIC METHANE:

IMPORTANCE OF MICROBIAL PHYSIOLOGY AND DIVERSITY

By

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Abstract

Recently, atmospheric CH_4 concentration has risen dramatically, apparently due to human activities. Since CH_4 is involved in several atmospheric processes that regulate Earth's climate, it is important that we understand the factors that control its atmospheric concentration. One such factor is biological CH_4 consumption in well–drained soils. Although this sink may comprise nearly one–tenth of the annual destruction of atmospheric CH_4, we know relatively little about it. I conducted a research project to investigate the influences of CH_4 supply, soil moisture, dissolved salts, and NH_4^+–fertilizer on the activity of soil CH_4 oxidizers.

When starved of CH_4, two upland taiga soils gradually lost their capacities to oxidize CH_4, indicating that the process was not merely fortuitous, and that the organisms involved were truly methanotrophic. The relationship between soil moisture and CH_4 consumption was parabolic, with maximum oxidation occurring at a moisture level that achieved the maximum possible CH_4 diffusion rate, while minimizing water stress on the methanotrophs. Optimal soil moisture occurred in a relatively narrow range among an array of physically dissimilar soils, providing that moisture content was expressed as a percentage of the water holding capacity for a particular soil, rather than as absolute water content.

In recent years, one of the most intensely investigated controls on soil CH_4 consumption has been its inhibition by NH_4^+–fertilizer. In addition to NH_4^+, however, I found that other ions inhibited CH_4 oxidation. In some soils non–NH_4^+ ions were so toxic that they completely masked the NH_4^+ effect. It is crucial, therefore, to control for salt effects when investigating NH_4^+–inhibition. In both field and laboratory experiments, CH_4 consumption in a birch soil was sensitive to NH_4^+, whereas a spruce soil was unaffected. In the birch soil, NH_4^+ apparently inhibited methanotroph growth, rather than enzymatic CH_4 oxidation, whereas methanotrophs in the spruce soil were apparently insensitive to NH_4^+. These results

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suggest that the primary landscape-level control over the response of soil CH_4 consumption to NH_4^+-fertilization is the cross-site distribution of physiologically distinct CH_4 oxidizers.

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Preface

The research described herein was born of a desire to put the "bug" back in its natural surroundings. My early training was in general microbiology and microbial physiology. My physiological background was to be, I believe, the ideal underpinning for my expansion into microbial ecology. I began exploring the physiology of CH_4–oxidizing bacteria (methanotrophs) at the University of Texas at Arlington in 1990 when I started my M.S. project under the direction of Dr. Alan DiSpirito. While manipulating the axenic environment of a bench–top batch culture, I frequently contemplated the nebulous complexities of the natural environment, wondering what role these nutritionally unique organisms might play in the grander scheme of the real world. When I voiced this predisposition to Dr. DiSpirito, he suggested I contact Dr. Bill Reeburgh at the University of Alaska Fairbanks. I did, and was in Fairbanks six months later. Dr. Reeburgh soon left Alaska to join the faculty at the University of California, Irvine. Before leaving, however, he helped arrange my transfer to the laboratory of Dr. Josh Schimel, who became the major advisor for my Ph.D. project. Given the opportunity, I honestly do not believe I could have constructed better circumstances than those in which I found myself during my Ph.D. studies. The Department of Biology and Wildlife, Institute of Arctic Biology, and other units within the University of Alaska have provided a dynamic, stimulating research and teaching environment. The high caliber group of ecologists and other scientists at UAF, and the exceptionally good rapport between students and faculty, have prepared me, I believe, for a productive career in science education and research.

I extend heart–felt thanks to the faculty and students of the Department of Biology and Wildlife. In particular, I thank fellow graduate students Merav Ben–David, Loren Buck, Joy Clein, Paul Cotter, Jon Lindstrom, Susan Sharbaugh, and Mitch Wagener for intellectual and motivational support, and for sharing the

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ups and downs that graduate study inevitably affords. Foremost, I thank my wife, Cindy, also a fellow graduate student. The synergism between us has enabled me to be a better scientist, and citizen, than I could possibly be on my own. I also thank members of my Graduate Advisory Committee, Drs. Joan Braddock, Roger Ruess, Elena Sparrow, and Keith Van Cleve whose endless encouragement, valued advice, and constructive criticism have had an inestimable impact on my scientific and professional development. I extend special thanks to my major advisor, Dr. Josh Schimel, who has been an exemplary mentor and has provided unwavering support in every aspect of my graduate training, including intellectual, moral, and financial. Any professional success I have achieved, or might in the future, is in large part to his credit.

Several organizations funded my research and put food on my table for five years, including the National Science Foundation LTER program; the US Environmental Protection Agency; the US Department of Energy; the UAF Center for Global Change and Earth System Research; Sigma Xi, The Scientific Research Society; and the Department of Biology and Wildlife.

Chapters II through IV of this dissertation have been adapted from manuscripts submitted for publication in specialized journals. Hence, formatting varies among these chapters. My coauthors for the corresponding manuscript and the journal to which it has been submitted are footnoted on the first page of each chapter.

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I. Introduction

The concentration of atmospheric CH_4 has risen dramatically in the past two centuries, most notably during the 1980s, primarily due to human activities associated with industry and agriculture (Prather et al. 1995; Khalil & Rasmussen 1990). This trend has drawn attention from scientists and policy makers because CH_4 affects atmospheric chemistry and physics. As a greenhouse gas, CH_4 is 21 times more potent than CO_2 at trapping infrared radiation (Shine et al. 1995). It also regulates concentrations of hydroxyl radical, an important mediator of atmospheric redox chemistry, and water vapor, a primary factor in cloud formation. Although it is difficult to predict the net effect of these combined factors, there is little debate that atmospheric CH_4 has the potential to affect Earth's climate (Dickinson & Cicerone 1986; Ramanathan et al. 1985; Thompson 1992). Hence, the international research community has identified as a critical research priority, developing the ability to predict how changing land use, atmospheric deposition, and climate will affect ecosystem methane fluxes (Schimel et al. 1992). As described in the IGAC (International Global Atmospheric Chemistry) program activity 7.2 (Melillo & Smith 1991), achieving this objective requires understanding the various processes mediating the global CH_4 cycle, including bacterial CH_4 oxidation in soils. Although this sink comprises 6–9% of the annual destruction of atmospheric CH_4 (Prather et al. 1995), relatively little is known about its function. Most research on this subject has emphasized either the regional quantification of soil CH_4 consumption, or the physiology of CH_4–oxidizing bacteria in pure culture. Little has been done to elucidate the ecosystem–level controls on CH_4 oxidizer activity. The purpose of the project described herein was to learn more about the ecology and physiology of soil CH_4 consumption and the microorganisms resposible for this process.

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CH_4–Oxidizing Microorganisms

Methane–oxidizing organisms have been known for almost a century (Whittenbury & Dalton 1981). Methanotrophs are a subgroup of the methylotrophs, a ubiquitous group of bacteria and yeasts that utilize reduced one–carbon (C_1) compounds, whether obligately or facultatively, as sole carbon and energy sources. Methylotrophs are physiologically and phylogenetically distinct from autotrophs, which utilize carbon dioxide as a carbon source (Anthony 1986). Unique among the larger group of methylotrophic bacteria are the methanotrophs, which are restricted to growth on methane (CH_4) as a C source, whereas other methylotrophs cannot use this compound (Anthony 1982; Tsuji et al. 1990).

Methanotrophs comprise an evolutionarily diverse group of strictly aerobic, Gram–negative bacteria (Tsuji et al. 1990). Although reports of facultative methanotrophs are not unusual (e.g., Patel et al. 1982; Patt et al. 1974; Zhao & Hanson 1984), many of the cultures so reported either lost their ability to use CH_4, were actually syntrophic mixed cultures, or were never confirmed as pure (Anthony 1982; Green et al. 1984; lidstrom 1992; Lidstrom–O'Connor et al. 1983; Tsuji et al. 1990). Hence, no confirmed examples of facultative methanotrophs are known, although some strains are artificially adaptable to growth on methanol (Davis et al. 1987; Hyder et al. 1979). Morphological characteristics vary widely among methanotrophs, many being rod–shaped, whereas others are coccoid or vibrioid; they may be motile or nonmotile, encapsulated or not. Many form resting stages including exospores and a variety of cysts. One unifying morphological characteristic, however, is that all methanotrophs possess complex intracytoplasmic membrane systems (Anthony 1982; Quayle 1972; Whittenbury et al. 1970). Two broad categories of methanotrophs are recognized based on the arrangement of these membranes and the biochemical pathway employed for C assimilation. Type I strains possess stacked internal membranes distributed throughout the cell and assimilate C through the ribulose monophosphate (RuMP) pathway. Type II cells contain concentric membranes lining the inner cell periphery and assimilate C via

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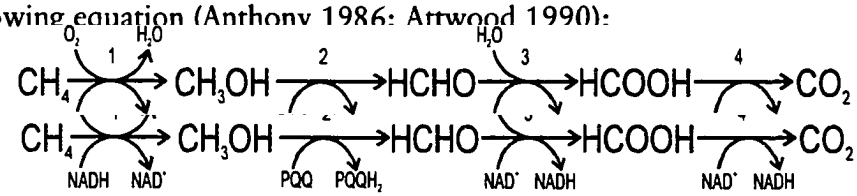
the serine cycle (Lidstrom 1992). Additionally, type I organisms lack α-keto-glutarate dehydrogenase in the tricarboxylic acid cycle, while type II strains retain the complete cycle (Anthony 1982).

All methanotrophs oxidize CH_4 through a four–step process according to the following equation (Anthony 1986; Attwood 1990):



The reactions are catalyzed by (1) methane monooxygenase, (2) methanol dehydrogenase with a pyrrolo–quinoline quinone prosthetic group (PQQ), (3) formaldehyde dehydrogenase, and (4) formate dehydrogenase.

Methanotrophs are of global geochemical importance. These ubiquitous organisms contribute to N cycling through nitrogen fixation, fortuitous nitrification, and assimilation of nitrate and ammonium (de Bont & Mulder 1974; Jones & Morita 1983; Topp & Knowles 1984; Wolfe & Higgins 1979). Relative to typical heterotrophs and nitrifiers, however, methanotrophs are probably insignificant to the global N cycle. Methanotrophs are, however, key participants in C cycling. Biological methane production is the primary source of atmospheric methane. Approximately 350 Tg (1 Tg = 10^{15} g) of methane are produced annually by anaerobic bacteria (methanogens). Only a fraction of this gas reaches the atmosphere, however, due to the activity of methanotrophs, which provide the sole biological means of cycling methane back to carbon dioxide (Anthony 1982; Higgins et al. 1981; Tsuji et al. 1990; Wolfe & Higgins 1979).

It is important to note that most of the information discussed to this point has been acquired from studies of methanotrophs in pure cultures isolated by enrichment with CH_4 concentrations of 5–50%. Although the soil CH_4 sink is biological, we are not certain whether the responsible organisms are similar to those known from pure–culture work, as no pure cultures exhibit CH_4 oxidation kinetics similar to those observed in well–drained soils (Bender & Conrad 1992, 1993).

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Sources and Sinks of Atmospheric CH_4

Atmospheric CH_4 concentration is predominantly driven by the net balance between biological production by methano*genic* bacteria and oxidation by methano*trophic* bacteria (Wolfe & Higgins 1979). It has been estimated that at least 90% of all biologically produced CH_4 fails to enter the atmosphere because it is intercepted by methanotrophs (Chanton & Dacey 1991; Galchenko et al. 1989; Yavitt et al. 1988). Of the forces driving atmospheric CH_4 concentration, production is relatively well characterized (Fung et al. 1991; Miller 1991; Bartlett & Harris 1993) compared with soil consumption. Uncertainties remain, however, on both ends of the cycle (Fung et al. 1991).

I will provide only a brief overview of sources and sinks of atmospheric CH_4 (for detailed reviews, see Fung et al. 1991; Miller 1991; Bartlett & Harris 1993; Prather et al. 1995). The following values represent current "best estimates" (Prather et al. 1995). Total annual CH_4 release to the atmosphere is estimated to be 535 Tg, with natural sources comprising about 30%, and anthropogenic sources about 70% of the total. The predominance of anthropogenic sources is indicative of the rapid rise in atmospheric CH_4 in the past two centuries. Identified natural sources can be broadly categorized (the parenthetical values represent terragrams, or 10^{15} g): wetlands (115), termites (20), oceans (10), and other (15), which includes natural–gas seeps. Anthropogenic sources include fossil fuel combustion (100), and biogenic sources (275), which include agriculture, biomass burning, and human and animal waste.

Total annual destruction of atmospheric CH_4 is estimated at 515 Tg, most of which (485) is chemically oxidized in the upper atmosphere by OH radical. The remaining portion (30) is consumed by soil bacteria. Although the soil sink may seem small, its magnitude is similar to the estimated annual increase in the atmospheric CH_4 pool (37) throughout the 1980s. Thus, significant reductions in this sink would result in significantly larger annual increases in atmospheric CH_4. In the past three to four years, the growth of atmospheric CH_4 apparently has

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declined due to decreased anthropogenic emissions in temperate latitudes (Dlugokencky et al. 1994). Because human activities exert great regional influence on soil CH_4 consumption (Keller et al. 1990; Ojima et al. 1993), it may be particularly useful at this time to preserve and, if possible, enhance the soil CH_4 sink through reclamation of disturbed ecosystems. Finally, it is important to realize that the dominant sink, atmospheric OH, is under attack by many reductive compounds, including CH_4, released into the atmosphere by human activity. As the concentrations of these compounds increase, OH is consumed, potentially diminishing its strength as a CH_4 sink (Thompson 1992; Thompson & Cicerone 1986). By contrast, the strength of the soil sink may actually increase in response to elevated CH_4 supply (Schütz et al. 1990), particularly if the responsible organisms are sustained by atmospheric CH_4. Thus, if the current soil sink represents 6% of the total, and its magnitude increases as the hydroxyl sink decreases, then the soil sink could provide an increasingly important negative feedback to future increases in atmospheric CH_4. Moreover, there is evidence that the soil sink is currently important: Ojima et al. (1993) estimated that without the CH_4 sink attributed to temperate soils alone, the rate of atmospheric increase would be 50% higher.

Soil CH_4 Consumption

Soil consumption of atmospheric CH_4 has been recognized for a relatively short time. Since it was first reported in 1982 (Harris et al. 1982), it has been observed in all major terrestrial biomes, including alpine and arctic tundra (Neff et al. 1994; Whalen & Reeburgh 1990); taiga, temperate and tropical forests (Keller et al. 1990; Steudler et al. 1989; Whalen et al. 1991); boreal and temperate wetlands; and temperate and tropical grasslands (Bartlett & Harris 1993). With few exceptions, methane consumption rates in upland soils are surprisingly constant (1–2 $mg \cdot m^2 \cdot d^{-1}$) across diverse ecosystems, which points to methane supply as the primary, and most distal control on consumption (Schimel et al. 1993). There is

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evidence, however, that natural and anthropogenic disturbances can significantly reduce CH_4 consumption rates in soil (Ojima et al. 1993; Keller et al. 1990).

Fertilization, atmospheric N–deposition, and conversion of natural ecosystems to agriculture are known to inhibit CH_4 consumption by up to 65% in temperate and taiga forests (Fig. 1; Castro et al. 1995), and up to 75% in temperate grasslands (Mosier 1991) and moist tropical forest soils (Keller et al. 1990). Fertilization is the best documented, but any disturbance that alters the soil chemistry, moisture regime, or physical structure can diminish CH_4 consumption (Hansen et al. 1993; Hütsch et al. 1994; Keller et al. 1990; Ojima 1993; Topp 1993). Inhibition dynamics vary tremendously, however, and do not appear constrained by geographic or climatic factors. Various ecosystems within the same region may respond differently to disturbance. For instance, CH_4 consumption in a taiga birch forest was inhibited by 65% after 5 years of annual fertilization, but a nearby spruce stand in the same successional sequence exhibited no response to a similar fertilization regime (Fig. 1). Even in the birch site, however, inhibition did not occur until the second year of fertilization, whereas soils in other ecosystems were immediately sensitive (Chapter V; Crill et al. 1994; Schnell & King 1994; Hansen et al. 1993). It is not known why responses to similar disturbances affect soil CH_4 consumption differently in various ecosystems.

Project Objectives

Through time, as more and more natural lands are disturbed for human use, there is strong potential for human activity to reduce the soil CH_4 sink, and, in turn, affect atmospheric CH_4 concentration. Hence, an improved understanding of the mechanisms by which disturbances inhibit soil CH_4 consumption is needed. Upland forests and grasslands are typically well–drained and have high evapotranspiration potentials. These conditions favor atmospheric CH_4 consumption by promoting free diffusion of atmospheric CH_4 and O_2 into the soil. Available NH_4^+ is usually low in mature ecosystems, as well, which may further

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promote methanotrophy by avoiding NH_4^+–inhibition. When such ecosystems are cut, burned, ploughed, irrigated, or fertilized, CH_4 consumption may be diminished due to excess NH_4^+ availability or restricted gas diffusion, whereas CH_4 production may be enhanced by increased anoxia and nutrient availability.

The general purpose of this project was to investigate two of the most prominent disturbance–related controls on CH_4 consumption, soil moisture and NH_4^+ fertilization, and also to investigate the type(s) of organisms that oxidize atmospheric CH_4, since their physiological responses to disturbance form the biological underpinnings of a diminished soil CH_4 sink. The project had five specific objectives:

1. describe the relationship between soil moisture and CH_4 consumption;
2. determine whether soil microorganisms use atmospheric CH_4, and particularly, whether they use it for growth (not merely maintenance);
3. determine the effects of dissolved salts on CH_4 oxidation;
4. determine the mechanism of NH_4^+–inhibition of CH_4 oxidation;
5. explain cross–site variation in NH_4^+–inhibition dynamics.

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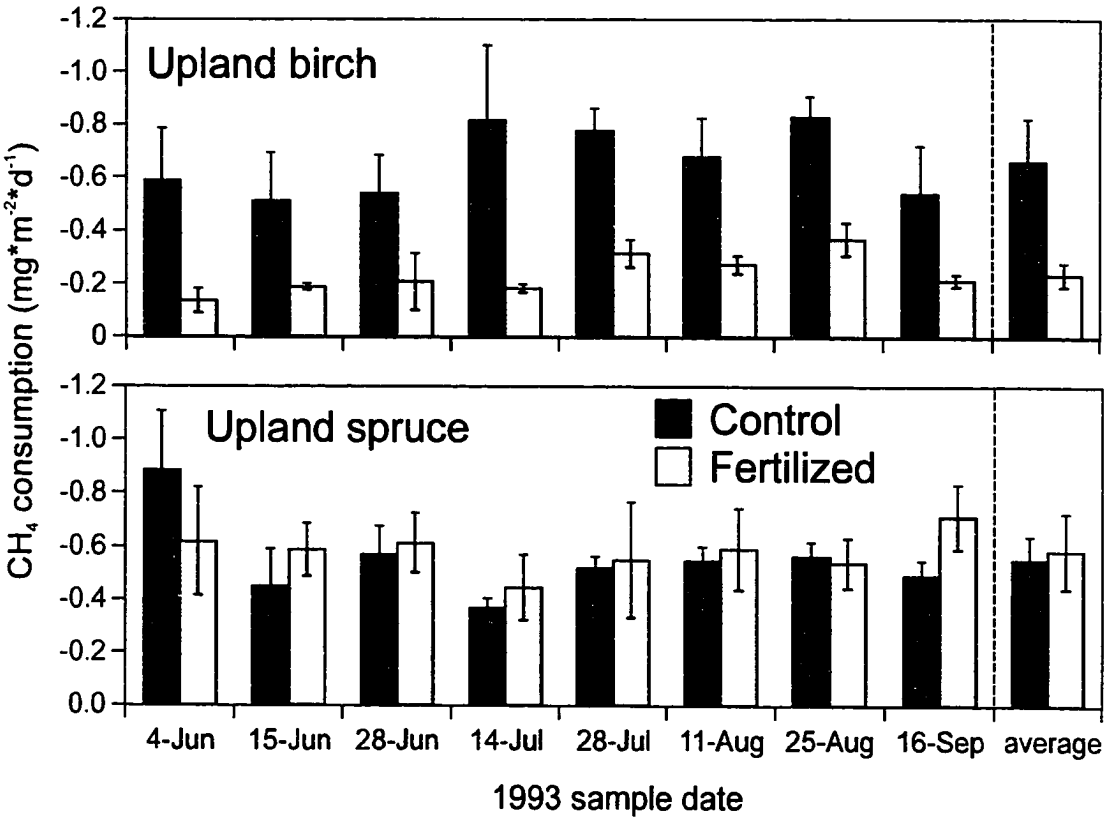

Figure 1. Effect of annual NH_4^+ fertilization on CH_4 consumption in two upland taiga soils. NH_4NO_3 was added each June from 1990–1993.

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II. Effect of CH_4–Starvation on CH_4–Oxidizing Organisms in Upland Taiga Soils[†]

Biological CH_4 consumption from the atmosphere in well–drained soils is estimated to comprise 3% to 9% of the global atmospheric CH_4 sink (6). Although this process plays a key role in global CH_4 cycling (8), little is known about the organisms involved (7). Although, methanotrophic bacteria have been studied for many years, CH_4 oxidation kinetics resembling those in upland soils have never been observed in pure cultures (2, 3). Hence, it is unclear whether the soil CH_4 consumers closely resemble those known from pure–culture studies. Furthermore, the types of organisms involved may vary from one site to another. For instance, Steudler et al. (12) concluded that CH_4 was oxidized by methanotrophs in nonnitrifying soils, but by nitrifiers in nitrifying soils. Gulledge et al. (5; Chapters IV & V) found that CH_4 consumption responded differently to NH_4^+ and salt additions in two taiga forest soils, indicating physiologically distinct CH_4 oxidizer populations. Thus, soil CH_4 consumption may be carried out by one or more of a physiologically heterogeneous group of organisms. Whether the organisms involved use CH_4 for energy or C, or whether it is a fortuitous side reaction as thought to occur with nitrifiers is uncertain (1). Recently, Schnell and King (11) concluded that CH_4 consumers in a temperate forest soil in Maine rely on atmospheric CH_4 for maintenance, suggesting the responsible organisms are methanotrophs. We conducted a similar experiment to determine whether the CH_4–oxidizing organisms in two upland taiga soils required atmospheric CH_4 to maintain their ability to oxidize CH_4.

[†]J. Gulledge & J.P. Schimel. Submitted to *Applied & Environmental Microbiology*

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Materials & Methods

Soils were sampled in a birch (*Betula papyrifera*) stand and a white spruce (*Picea glauca*) stand in the Bonanza Creek Experimental Forest in interior Alaska approximately 25 km southwest of Fairbanks. These sites were established in 1987 as part of the U.S. Long–Term Ecological Research program (LTER). Both are on south–facing slopes, well–drained, and underlain by a silty, micaceous loess. These sites represent distinct stages of a post–fire successional sequence. The birch and spruce sites are approximately 80 and 200 years post–burn, respectively. More detailed descriptions of the sites can be found in Gulledge et al. (5; Chapter V).

Triplicate 7.6×10–cm mineral soil cores were taken from each site. The forest floor was removed prior to coring. For transport to the laboratory, cores were stored in perforated plastic bags to allow free gas exchange with the atmosphere. Each core section was homogenized by sieving through a 4 mm screen. Water holding capacity was determined gravimetrically and soil moisture was adjusted to 30% of water holding capacity, a previously determined optimum for CH_4 oxidation (Chapter III). Duplicate samples (10 g dry weight) from each of the three cores were placed in separate 70–ml serum vials. One sample from each core was deprived of CH_4 while the other served as a control. This design yielded three replicates from each site for each experimental treatment.

To deprive the samples of CH_4 without allowing oxygen depletion or CO_2 accumulation, a continuous–flow apparatus was used. The serum vials were fitted with butyl rubber stoppers and connected in series using 3.2–mm o.d. copper tubes connected with 3.2–mm i.d. Tygon tubing. This design ensured uniform gas flow through all the vials within a series. The birch and spruce samples were placed in separate series so that neither would be exposed to exotic gaseous products, and to avoid possible cross–inoculation of microorgan-isms. CH_4–starved samples were continuously supplied with compressed Ultra Zero air (Scott Specialty Gases, Plumsteadville PA). Control samples were continuously supplied with laboratory air

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by applying a vacuum to the outflow side of the samples. The flow rate was maintained independently through each series of vials at 2 $L \cdot h^{-1}$. This flow rate was sufficient to prevent detectable CH_4 depletion in the control samples. In an attempt to minimize moisture loss over the duration of the experiment, inflowing air was humidified by bubbling through deionized water.

Soil samples were repeatedly incubated for (approximately) 2–week intervals over 11 weeks. At the end of each starvation interval, all samples were removed from the gas–flow system, flushed with laboratory air (~1.9 ppm CH_4), sealed, and assayed for CH_4 oxidation capacity. Because the two soils oxidized CH_4 at different rates, the assay period was 24 h for the birch soil and 2 h for the spruce soil. CH_4 oxidation rates were determined by difference in CH_4 concentration in the headspace at the beginning and end of the assay period. A two–point rate measurement was used because previous work had shown that CH_4 oxidation rates were linear with respect to CH_4 concentration (first–order kinetics) over the course of the incubation period. At the end of the assay, samples were replaced on the gas flow system, thereby initiating a new starvation interval. The experiment was terminated after 80 days because the flow system failed.

Rank–transformed data were analyzed by 2–way repeated measures ANOVA using sampling date and CH_4 treatment as independent factors.

Results & Discussion

Throughout the experiment, CH_4 was maintained at a mean (±SE) of 0.32 ± 0.039 ppm in the CH_4–starved samples, a concentration similar to the oxidation thresholds in these soils (unpublished data). In the control samples, CH_4 was maintained at 1.93 ± 0.036 ppm. Initial CH_4 oxidation rates were about 10 times lower in the birch soil than in the spruce soil (Fig. 1). We have observed this difference in oxidation capacity between these two sites repeatedly over several years. In the birch soil, CH_4–starvation ultimately resulted in a decrease of 42% in

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CH_4 oxidation capacity relative to controls over the course of the experiment ($p=0.036$). Inhibition was not apparent after the first starvation interval, during which, CH_4 oxidation capacity decreased in both the control and starved samples. Thereafter, rates in the control samples leveled off, while the starved samples continued to lose oxidation capacity. In the spruce soil, starved samples were eventually inhibited by 68% ($p=0.003$). Control rates remained relatively constant throughout the experiment, while the rates in starved samples declined steadily throughout. Moisture steadily declined in all samples, but there was no difference between starved and control samples ($p=0.64$). Thus, the difference in oxidation capacity between starved and control samples was due to CH_4 supply.

What type(s) of organisms consume atmospheric CH_4, or whether this activity is beneficial or fortuitous is uncertain (2, 3, 7, 10). It is commonly assumed that methanotrophic bacteria similar to those known from pure culture studies are involved (7, 10). In some soils, prolonged exposure to elevated CH_4 supply increases the oxidation capacity, indicating that there is methanotrophic potential. The half–saturation constants (K_s) of CH_4 uptake in well–drained soils, however, are typically orders of magnitude lower than those observed in pure cultures (2, 3, 4, 13). Thus, it has been speculated that atmospheric CH_4 oxidizers represent distinct populations from those responding to higher CH_4 concentrations (2, 11).

Based upon varied responses of soil CH_4 consumption to N–fertilization, there appear to be at least three distinct physiological groups of atmospheric CH_4 oxidizers in upland taiga soils (Chapter V). It is not clear, however, whether these differences result from fundamentally different types of bacteria, or merely subtly different populations of methanotrophs. The soils examined in the current study responded differently to N additions (Chapter V), yet the present results show that the CH_4 oxidizers in both soils relied on atmospheric CH_4 to maintain oxidation capacity. These data indicate that the enzymes involved are dedicated to CH_4 oxidation, and do not cometabolize CH_4 as a side reaction, as presumed for

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nitrifying bacteria (1). If the reaction were fortuitous, the absence of CH_4 should not have diminished the CH_4 oxidation potentials. Thus, the responsible organisms are most likely strict methanotrophs. It is also possible, however, that they are facultative CH_4 oxidizers using an alternative enzyme system during CH_4 starvation (9). Working with a temperate forest soil exhibiting a different N response than our taiga soils, Schnell and King (11) also observed reduced CH_4 oxidation capacity due to starvation, and concluded that methanotrophs were probably responsible.

It is important to identify the true nature of the soil organisms responsible for atmospheric CH_4 consumption, since this has been uncertain and physiological differences have been observed. Nitrifiers appear to be important in soils that nitrify intensely (12). In most soils studied, however, atmospheric CH_4 consumption appears to be fundamentally methanotrophic.

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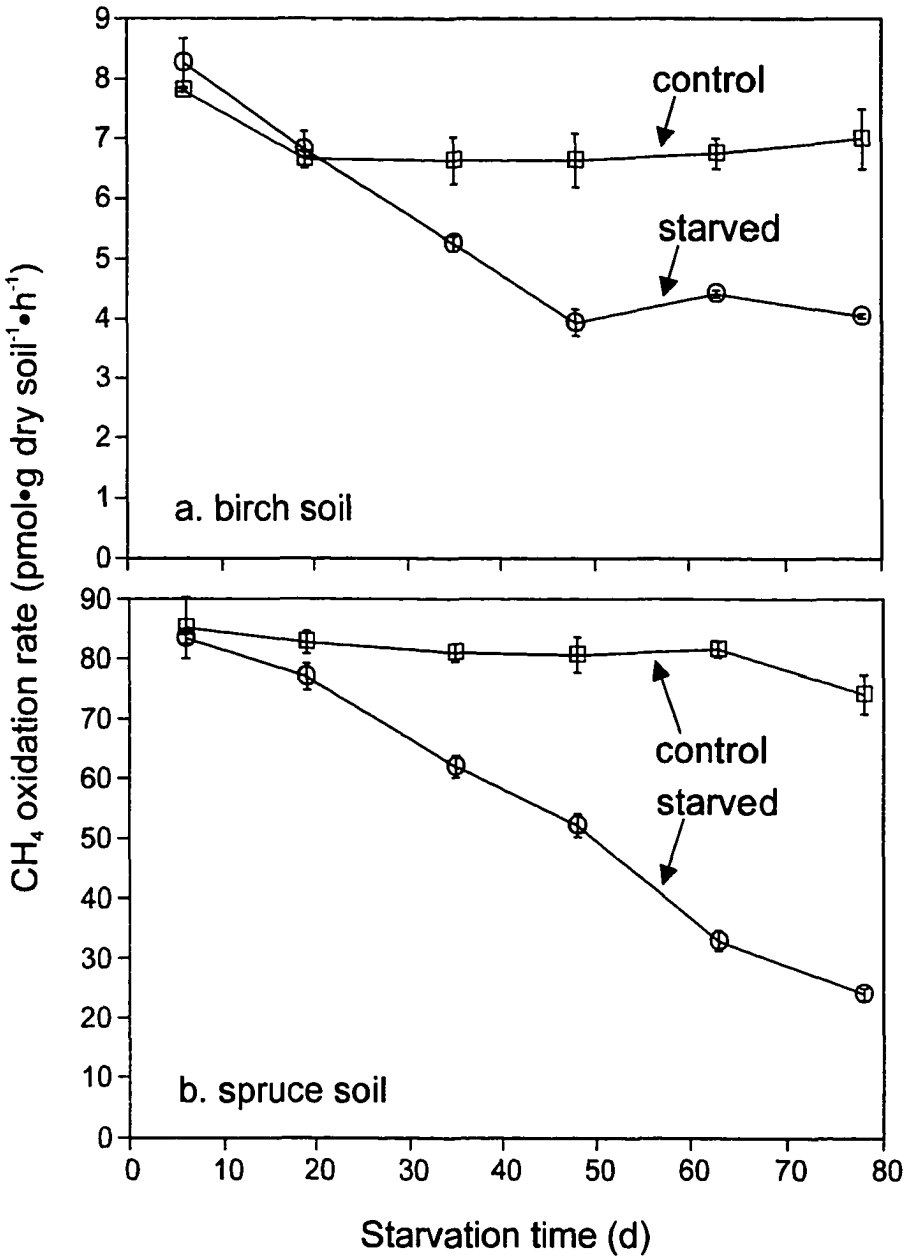

Figure 2. The effects of CH_4–starvation on CH_4 oxidation capacity in two upland taiga soils.

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III. Moisture Control over Soil CH_4 Consumption: A Unifying Measure Across Sites†

Upland soils comprise an estimated 6% of the global atmospheric CH_4 sink, and natural wetlands emit an estimated 21% of the global annual source (Prather et al. 1995). Approximately 34% of all wetland CH_4 efflux occurs in northern latitudes (>45°N; Bartlett & Harris 1993). The rate of atmospheric CH_4 consumption in soil is limited primarily by gas diffusion, such that consumption is favored by decreasing soil moisture (Striegel 1993). Conversely, microbial activity is sensitive to water stress. Hence, the optimum soil moisture for CH_4 consumption balances gas diffusion with sufficient water potential in order to maximize CH_4 supply while minimizing water stress on the soil methanotroph community.

Climate models predict warmer, drier conditions in the Arctic due to global warming (Manabe & Wetherald 1987; Wilson & Mitchell 1987). Under such conditions, some arctic wetlands have the potential to convert from net CH_4–producing to net CH_4–consuming systems as water tables drop and the depth of thawed, aerated soil increases (Whalen & Reeburgh 1990). In uplands, however, drying could suppress CH_4 consumption rates due to increased water stress on soil microbes. In the taiga forests of interior Alaska, increased summer precipitation is expected to accompany warmer conditions (Schlesinger & Zhao 1989; G. Weller, pers. comm.). Wetter forest soils may result in slower CH_4 consumption due to decreased diffusion rates. In order to model changes in CH_4 consumption under changing moisture regimes, a simple, quantitative relationship between CH_4 consumption and soil moisture is desirable.

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In relation to soil moisture, the two primary controls on atmospheric CH_4 consumption are diffusion, a direct function of water–filled pore space (WFPS), and soil water potential. Although soil moisture typically is expressed in absolute terms (g H_2O•g dry $soil^{-1}$), the quantitative relationships among absolute water content, water–filled pore space (WFPS), and water potential vary widely among physically different soils. Hence, absolute expressions of water content cannot adequately relate optimum moisture for CH_4 consumption across sites, or studies, with soils of different textures. Expressions of soil moisture that normalize the relationship between moisture and CH_4 consumption across sites are therefore desirable.

Percent of water holding capacity (%WHC) is a relative measure that expresses moisture as a proportion of the mass of water required to saturate a quantity of dry soil. This measure is used often in studies of soil microbial activity, and the optimum for microbial respiration across diverse soils appears relatively constant near 60% WHC (Linn & Doran 1984). In many soils %WHC closely parallels %WFPS (Linn & Doran 1984) and may therefore provide a convenient index of diffusivity and water potential across a range of physically different soils. The advantages of this technique over WFPS determination are ease of measurement, accuracy, and effectiveness on disturbed samples.

We investigated the relationship between CH_4 consumption and soil moisture expressed as %WHC in five taiga and tundra soils of varying textures and organic matter content (Table 1). Our objectives were 1) to determine whether %WHC would provide a uniform measurement of optimum soil moisture content for atmospheric CH_4 consumption across sites, 2) to determine the range of %WHC most favorable to CH_4 oxidation, and 3) to describe the response of soil CH_4 oxidizers to decreasing water potential.

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Materials & Methods

The soils used in this study included three upland soils, birch taiga, spruce taiga, and dry heath tundra, and two wetland soils, wet meadow taiga and wet meadow tundra (Table 1). Tundra soils were collected at Toolik Lake Research Station in July 1993. Samples were transported to the University of Alaska Fairbanks and assayed within 1 week of collection. Taiga soils were collected from the Bonanza Creek Experimental Forest 20 km south of Fairbanks in June and July 1995. The upland taiga soils were processed and assayed within 4 days of collection, whereas the wet meadow taiga soil was stored at 12°C for 2 weeks before it was assayed. Due to limited equipment and facilities, only a limited suite of assays was performed on the two tundra soils, as described below.

The upland soils were homogenized by sieving through a 4–mm mesh screen. The wet meadow soils were homogenized by hand due to their fibrous, rooty texture; woody material and live roots larger than approximately 0.5–mm diameter were removed and the fibrous soil matrix was pulled apart. Water holding capacity was determined gravimetrically for each soil (Table 1). Samples were then adjusted to six different water contents: 10, 20, 30, 40, 50, and 60% of WHC. The two wet meadow soils and the dry heath soil were subsampled (5 g dry weight) into triplicate 235 ml Mason jars fitted with red butyl septa for syringe sampling of the headspace. The two upland taiga soils were subsampled (10 g dry weight) into triplicate 70 ml serum vials fitted with red butyl septa and sealed with crimped aluminum caps. CH_4 oxidation rates were determined in each sample by the difference in headspace CH_4 concentration at the beginning and end of an assay period. Similarly, carbon mineralization was determined (only in the three taiga soils) by the change in headspace CO_2 concentration over the assay period. This period varied among the five soils (0.5 to 24 h), depending on the oxidation capacity of each soil. Gas concentrations were measured with a Shimadzu GC–14A gas chromatograph equipped with a 2–m stainless steel column (3.2–mm o. d.)

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packed with Porapak Q resin, and flame–ionization (CH_4) and thermal conductivity (CO_2) detectors.

Following the oxidation assay, water potential was determined for all samples of the three taiga soils using an HR33T dew–point microvoltmeter with a C–52 sample chamber (Wescor, Inc., Logan UT). Actual soil water content was confirmed in the taiga soils by oven–drying at 60°C. Finally, organic matter content was determined in the taiga soils by combustion in a muffle furnace for 48 hours at approximately 400°C.

Results & Discussion

Maximum CH_4 oxidation rates varied by about two orders of magnitude among the five soils (Fig. 3). CH_4 oxidation exhibited a parabolic response to increasing moisture. All soils exhibited an optimum moisture between approximately 20 and 40% WHC (Fig. 3), with a mean optimum near 35% (Fig. 4). This value was considerably lower than the optimum for respiration in the taiga soils, which was >40% WHC in all cases (Fig. 4, 6b). The wet meadow soils exhibited a flatter response to moisture than the upland soils (Fig. 3, 6a), indicating that CH_4 consumption was generally less sensitive to both water stress and excess moisture in the wetland soils.

In those soils assayed for water potential, the optimum for CH_4 oxidation was between -0.02 to -0.35 MPa, whereas the optimum range for C mineralization was narrower (-0.02 to -0.11 MPa; Fig. 5). Not surprisingly, the two silty upland soils responded similarly to water potential; CH_4 oxidation rates dropped precipitously above -0.15 MPa and gradually below -0.35 MPa (Fig 5a,b). C mineralization was maximal at -0.11 MPa in both soils, and dropped precipitously below this value. In the wet meadow taiga soil, however, CH_4 oxidation was maximal at -0.05 MPa (Fig. 5c). This soil also maintained 70% of the maximum CH_4 oxidation rate at -0.50 MPa, compared with only 20–30% of maximum in the upland soils.

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Additionally, C mineralization in the wet meadow taiga soil did not decrease as dramatically as in the upland soils as water potential dropped. The response of CH_4 oxidation and C mineralization were nearly identical in this soil (Fig. 5c). The reason for the differences between the upland and wet meadow soils can be seen in Fig. 6. At a given degree of saturation, the wet meadow taiga soil had a slightly higher water potential than the upland taiga soils. Given the dramatically different physical characteristics of the wetland soil, however, it is perhaps surprising that the difference in response was not more dramatic.

In general, the right tails of the oxidation curves in Fig. 3 are controlled by diffusion, which limits CH_4 supply as soil moisture increases, whereas the left tails are determined by water potential, which inhibits biological activity with drying. The peak of the oxidation curve represents the most favorable balance between the physical and biological controls over CH_4 consumption. In some soils, diffusional limitations force methane oxidizers to function optimally at water potentials below those generally preferred by soil microbes, as indicated by the disparity between CH_4 oxidation and bulk soil respiration (Fig. 4). Although the optimum degree of saturation was relatively constant, there was more variability between soils at the wet and dry extremes of the oxidation curves (Fig. 4, 6a). This is apparent in that, individually, the moisture response curves in Fig. 3 fit a 2° polynomial function more tightly (r^2=0.77 to 0.97; data not shown) than the pooled data for all soils, when normalized by transformation to the % of maximum CH_4 oxidation (r^2=0.49; Fig. 4).

The unifying power of expressing soil moisture as %WHC is illustrated in Fig. 6. Percent WHC provided a relatively uniform prediction of optimum water content across diverse soil types for both CH_4 oxidation and microbial respiration, and also produced similar curves for the relationship between soil moisture and water potential. These data suggest, therefore, that %WHC is a good index of both WFPS and water potential in physically diverse soils. In the soils tested here, 20%

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WHC was the minimum soil moisture required to allow CH_4 oxidation to proceed without significant inhibition due to water stress, whereas 40% WHC was the maximum at which CH_4 oxidation proceeded without significant diffusional limitations (Fig. 3, 4).

Our results are in general agreement with those of Whalen and Reeburgh (1996), the only other study we know of that examined the relationship between %WHC and soil CH_4 consumption. They observed maximum consumption between 20–50% WHC in three different taiga soils. Czepiel et al. (1995) examined the relationship between CH_4 consumption and soil moisture in temperate grassland and woodland soils, and found that CH_4 consumption in individual samples responded parabolically to increasing moisture. There was a great deal of variability between samples, however, with regard to the optimum water content. Differences in organic matter content among the samples explained most of this variability. Since organic matter content is a major determinant of soil water holding capacity, our results suggest that plotting CH_4 consumption against %WHC may have removed much of the variation.

Our data suggest that CH_4 consumption in upland taiga soils of interior Alaska may be sensitive to climate change. Repeated observations have shown that field moisture in the two upland taiga soils we examined typically ranges between 20–40% WHC (unpublished data), which is also the optimum range for CH_4 consumption. Thus, either wetter or drier climate conditions could diminish the CH_4 sink in these soils. Conversely, peat soils, such as the wet meadow taiga soil, would be relatively insensitive to drying. Thus, these soils may be poised to provide a strong atmospheric CH_4 sink should dryer conditions prevail. This conclusion is similar to that of Whalen and Reeburgh (1990) relative to moist tundra soils.

It is important to note that the results presented here represent instantaneous responses of soil organisms to changes in the soil microclimate. This instantaneous response does not necessarily resemble the long–term adaptation of the soil

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ecosystem to climate change. Ecological succession of microbial communities will be determined by complex interactions among plant community dynamics, soil nutrient cycles, and soil temperature and moisture. It is impossible, therefore, to predict the long–term changes in soil CH_4 consumption without considering these factors. Even so, the instantaneous responses observed in short–term manipulations of soil moisture are useful for determining the quantitative impacts that changes in moisture, *per se*, will have on the potential of a soil microbial community to consume atmospheric CH_4.

Studies on a broader range of texturally different soils are needed to confirm our conclusions. Nonetheless, by effectively integrating two major controls on soil consumption of atmospheric CH_4, gas diffusion and microbial water stress, %WHC provides a convenient, unifying measure of soil moisture. The mean optimum value of 35% WHC is convenient for adjustment of soil moisture in laboratory experiments involving atmospheric CH_4 consumption, making comparison of results among different soils and across studies easier. More generally, %WHC appears to provide a convenient surrogate for water potential with regard to biological activities in soil. Finally, the relatively consistent 2° polynomial relationship between soil moisture and CH_4 consumption may be useful in developing predictive models of soil CH_4 flux under changing climate regimes. Such a model could be applied to different ecosystems without complex re–parameterization with regard to soil moisture.

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Table 1. Soil properties and site locations

soil	soil texture	% organic matter	water holding cap. (g H_2O•g dry $soil^{-1}$)
wet meadow tundra [a]	coarse, fibrous	85.5(1.1)[c]	7.2
wet meadow taiga [b]	coarse, fibrous	61.0(0.6)	4.7
dry heath tundra [a]	medium, grainy	58.4(7.7)[c]	2.5
upland birch taiga [b]	fine silty loess	5.42(0.10)	0.54
upland spruce taiga [b]	fine silty loess	6.68(0.06)	0.69

[a]Toolik Lake, foothills of the Brooks Range north slope, Alaska

[b]Bonanza Creek Experimental Forest, west ridge of the Tanana River valley, interior Alaska

[c]Taken from Nadelhoffer et al. (1991)

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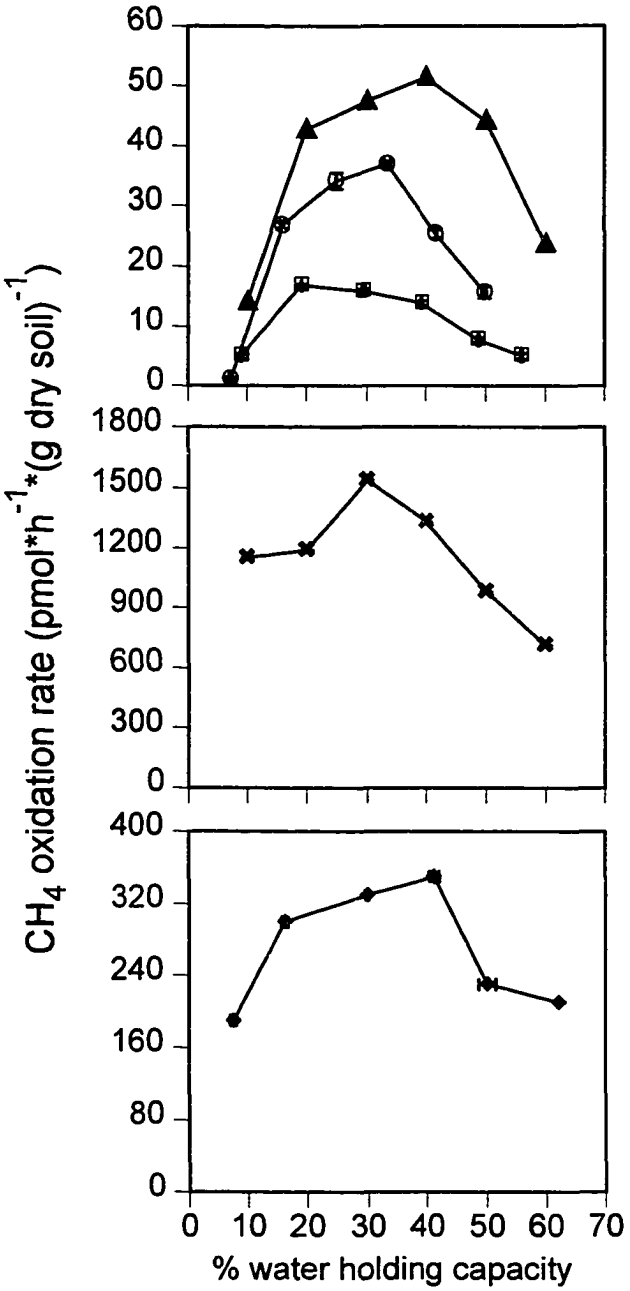

Figure 3. Relationship between CH_4 oxidation and soil moisture expressed as %WHC in dry heath tundra (▲), spruce taiga (O), birch taiga (□), wet meadow tundra (✖), and wet meadow taiga (◆) soils.

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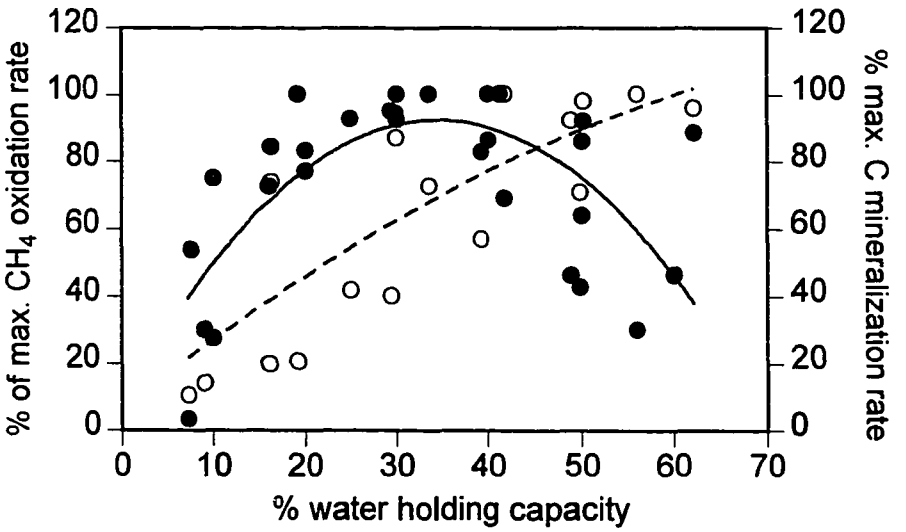

Figure 4. Mean response of CH_4 oxidation (●) and respiration (O) in the three taiga soils to changes in soil moisture expressed as %WHC. Data are fit to a 2° polynomial model.

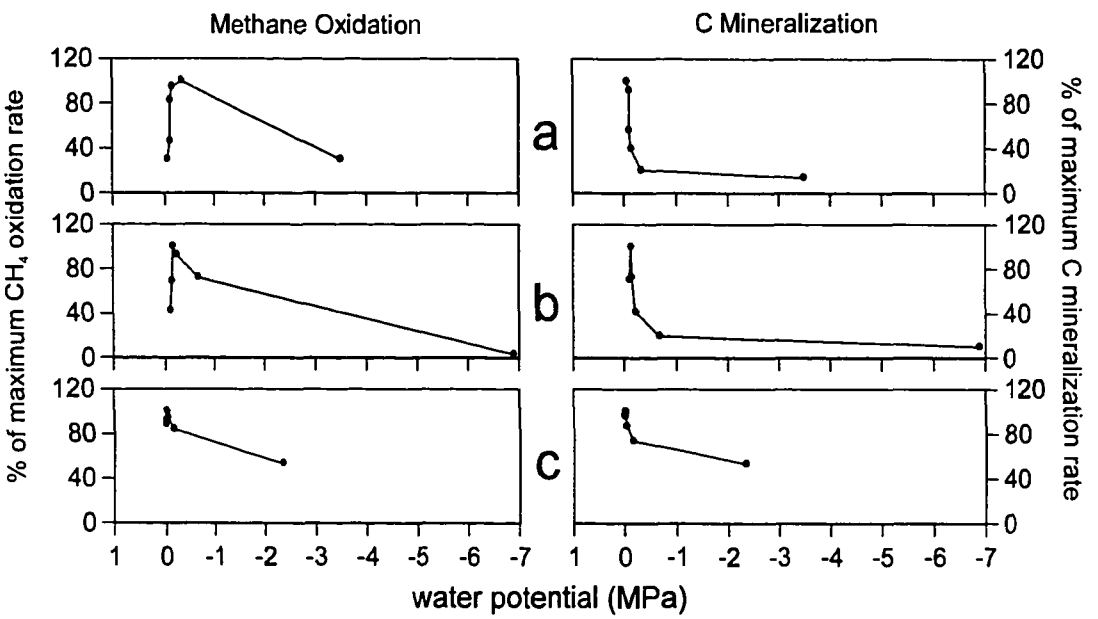

Figure 5. Relationship between microbial activity and water potential in (a) birch, (b) spruce, and (c) wet meadow taiga soils.

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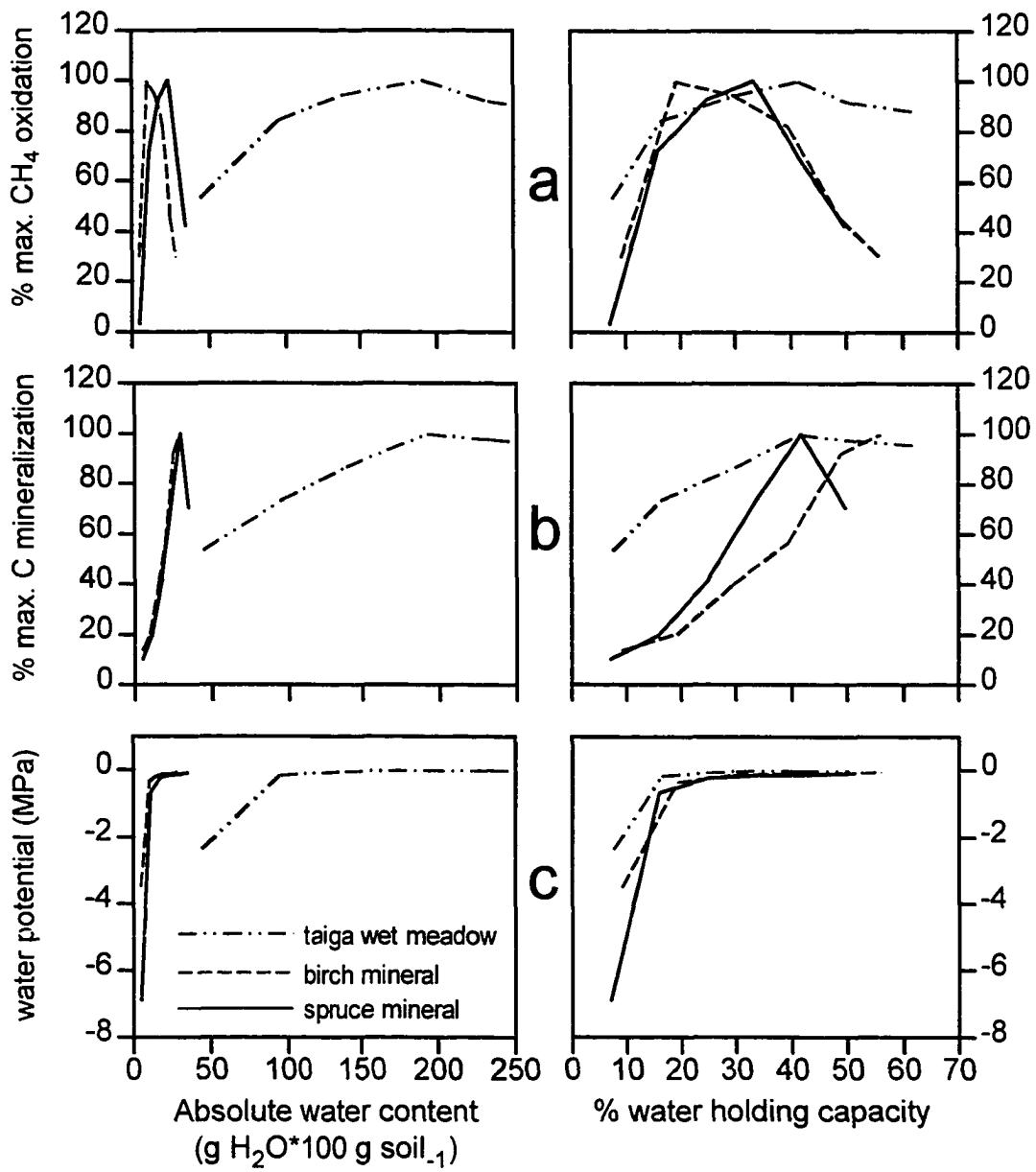

Figure 6. Different relationships of (a) CH_4 oxidation, (b) C mineralization, and (c) soil water potential to soil moisture expressed as absolute water content vs. % water holding capacity.

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IV. Salt and Ion Effects on Oxidation of Atmospheric CH_4 in Taiga and Tundra Soils[†]

Introduction

The role of soil CH_4 oxidation as a sink for atmospheric CH_4 has received much attention in recent years (28, 32, 41, 43, 44, 46). This sink is sensitive to human activity, and diminished CH_4 consumption due to N–fertilization has been one of the most extensively investigated disturbances (39). In efforts to understand NH_4^+–inhibition of soil CH_4 consumption, a number of researchers have examined the effects of NH_4^+ salts on CH_4 oxidation. Several different salts have been employed, including NH_4Cl, $(NH_4)_2SO_4$, and NH_4NO_3. Generally, researcher have assumed that the effects of these salts on soil CH_4 oxidation represent the effects of NH_4^+, *per se*. Hence, oxidation rates in the presence of an NH_4^+ salt often have been compared with rates in samples with only water added, allowing no control for either general salt effects or specific ion toxicities. In other cases, multiple salts have been employed, but without regard for the comparative properties of the ions involved.

There are at least two general mechanisms by which ions may inhibit microbial activity. First, if ion concentrations reach high levels, low water potential may inhibit a broad range of biochemical processes (22). Second, specific ions may disrupt the function of specific enzymes or membrane proteins (8, 11, 49). Specific ion toxicity is common, and may occur at concentrations much lower than those required to induce osmotic effects (5, 31, 34, 57). Cl^- ion is often toxic and is actively excluded by many bacteria (8, 9, 31). Cl^- has been noted for inhibiting specific intracellular processes (25, 34), bacterial growth and activity (30), and

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[†]J. Gulledge & J.P. Schimel. Submitted to *Applied & Environmental Microbiology*

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numerous biological soil processes (13, 14, 33). Na^+ also can be toxic and most bacteria selectively exclude it as well, even at the expense of maintaining large concentration gradients (18, 27, 36). By contrast, $SO_4^=$ and K^+ ions are relatively nontoxic to soil organisms (47, 50). For example, studies of soil nitrifier activity have found that both Cl^- and Na^+ inhibit nitrification to a greater extent than $SO_4^=$ and K^+ (13, 27). These relationships are not universal, however. Neither Cl^- nor $SO_4^=$ had much effect on the growth of *Rhizobium meliloti* (5), and in *Azospirillum brasilense*, $SO_4^=$ inhibited growth more severely than Cl^- (42). Hence, one cannot safely assume that a particular salt will or will not inhibit a particular bacterial process.

Researchers also apparently assume that the ion concentrations used in CH_4 oxidation studies are too low to induce osmotic effects, though the relationship between soil CH_4 uptake and osmotic potential is not well characterized (32), and should be considered a potential factor in salt–addition experiments. Moreover, since little research has examined the effects of salts on nonhalotolerant organisms it is possible that salt concentrations much lower than those typically considered inhibitory may affect soil CH_4 oxidizers. Soil drying experiments have indicated that soil CH_4 oxidation can be inhibited at water potentials as high as -0.4 MPa (21). Specific ion toxicities are also frequently ignored in CH_4 oxidation studies. There is ample evidence, however, that nonspecific and/or specific ion effects alter the CH_4 consumption capacities of soil samples. In a temperate forest soil, NH_4Cl inhibited CH_4 oxidation only slightly more than $NaNO_3$ (1), suggesting that either the osmotic potential of the soil solution or ion toxicity accounted for most of the observed inhibition. Similar results were observed in a landfill cover soil (26) and an agricultural humisol (15). Both NH_4Cl and KNO_3 inhibited *in situ* CH_4 consumption in peat soils to a greater extent than urea, despite the highest levels of extractable NH_4^+ occurring in the urea–amended plots (10). Similarly, CH_4 consumption in agricultural soils was inhibited by inorganic NH_4^+ fertilizer, but not

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by manure amendments producing comparable levels of extractable NH_4^+ (23, 58). Because the foregoing studies were not designed to investigate the effects of inorganic ions on CH_4 oxidation, it cannot be determined whether the observed effects are due to specific ion toxicities, the osmolarity of the soil solution, or both.

Given the mechanistic nature of several studies that have investigated the effects of NH_4^+ salts on soil CH_4 consumption, it is important to understand the effects that ions other than NH_4^+ have on the process. We studied the effects of $(NH_4)_2SO_4$, K_2SO_4, Na_2SO_4, NH_4Cl, and KCl on CH_4 oxidation in four Alaskan soils: two upland taiga forest soils, a wetland taiga soil, and an upland tundra soil. Our aim was to determine 1) whether the osmolarity of the salt solutions affect CH_4 oxidation, 2) whether certain ions are specifically toxic to soil CH_4 oxidizers, and, if so, 3) whether such toxicities mask potential NH_4^+ effects.

Materials and Methods

Field sites and sampling

Research was conducted using soils from four Alaskan sites. The primary study site was an upland birch (*Betula papyrifera*) stand located at the University of Alaska Fairbanks. The site is approximately 120 years post–burn, with an understory dominated by *Rosa acicularis* and *Equisetum* spp. The second taiga forest site was a mature white spruce (*Picea glauca*) forest located in the Bonanza Creek Experimental Forest on the west ridge of the Tanana River valley, approximately 25 km southwest of Fairbanks, and is part of the Bonanza Creek Taiga LTER project sponsored by the National Science Foundation. This site is approximately 200 years post–burn and has a near–continuous ground cover of feather moss (*Hylocomium splendens*). This site is described in greater detail elsewhere (20, 53). The upland tundra soil was from a shrub site dominated by dwarf birch (*Betula nana*), located near Toolik Lake on the north slope of the Brooks Range. The final site was a methane–producing wet meadow dominated by

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Carex and *Eriophorum* spp., located on the Tanana River floodplain in the Bonanza Creek Experimental Forest. The 4 soils examined represent a broad range of soil types (Table 2).

Salt treatments and assays

Soil was collected in bulk from the upper 10 cm of mineral soil in the three upland sites and the rooting zone of the wetland taiga soil. All soil from a particular site was pooled and homogenized by sieving through a 5–mm mesh screen, except for the wetland soil, which was processed differently due to its fibrous, rooty texture. Woody material and live roots larger than approximately 0.5–mm diameter were removed by hand and the fibrous soil matrix was pulled apart, leaving no soil clumps of more than 5 mm diameter.

Water holding capacity was determined gravimetrically, and moisture was adjusted to achieve a final water content equal to 30–35% of water holding capacity (Table 2) after final treatment with salt solutions. Parallel samples treated with deionized water were used to control for moisture effects. For the upland soils, each salt solution (0.1 ml•(g dry soil)$^{-1}$) was added to a single bulk sample, which was then mixed for approximately 2 minutes using an impeller attached to a hand–held power drill operated at 500 RPM. This technique successfully homogenized the fine–textured soils, which had a strong tendency to clump when wetted. Because of its coarse, organic texture, the wetland taiga soil was prepared differently. Individual samples (2 g dry wt.) were preweighed into 70–ml serum vials, and then a 0.5–ml aliquot of salt solution or deionized water was added to each vial. Each sample was manually homogenized by vigorous stirring. All samples were sealed and allowed to equilibrate overnight.

In the birch soil, salts were added at a rate of 5.6 μmol cation•(g dry soil)$^{-1}$, which represents approximately 80 mg–N•(kg dry soil)$^{-1}$ for the NH_4^+ salts. All salts were equinormal with respect to cation concentration. Samples (10 g dry

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weight) were placed in 70 ml serum vials and sealed with butyl rubber septa. Methane oxidation was measured in triplicate samples for each salt and deionized water treatment at several initial CH_4 concentrations: approximately 1.8, 5, 10, and 15 ppm. Headspace CH_4 concentration was adjusted by injecting an appropriate volume of 1% CH_4 premixed in air (Scott Specialty Gases, Plumsteadville PA). Three–hour oxidation assays were performed every 24 h for 3 days, and then every 48 h through the 9th day, or until a threshold concentration was established. An additional set of assays examined the Cl^- salts at a lower concentration, 0.85 μmol cation•(g dry soil)$^{-1}$, or about 12 mg–N•(kg dry soil)$^{-1}$. Three CH_4 concentrations were used: 1.8, 6, and 10 ppm. CH_4 was analyzed by gas chromatography with flame ionization detection as described by Gulledge et al. (20), and using a sampling apparatus as described by Breitenbeck (6).

Soil water potential was determined in separate, duplicate samples for each salt treatment (only at the higher salt concentration) and deionized water. Water potential was measured using an HR33T dewpoint microvoltmeter with a C–52 sample chamber (Wescor, Inc., Logan UT).

A limited set of assays was performed in the spruce, tundra, and wetland soils. K_2SO_4, $(NH_4)_2SO_4$, KCl, and NH_4Cl were examined at a final cation concentration of 3.4 μmol•(g dry soil)$^{-1}$, or about 50 mg N•(kg dry soil)$^{-1}$. Samples were prepared as above, except for the shrub tundra soil, which was incubated in 235–ml Mason jars, and whose moisture content was not altered from field content. For each soil, a single oxidation assay was performed at 10 ppm CH_4. Incubation times varied with regard to the oxidation capacity of each soil.

Statistical Analyses

The effects of salts and CH_4 concentration on CH_4 oxidation in the birch soil were analyzed by ANCOVA with salt treatment as the independent factor and initial CH_4 concentration as the covariate. In this case, Bonferroni contrasts were

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used for multiple comparisons. Water potentials and salt effects on CH_4 oxidation in soils examined at a single CH_4 concentration were analyzed by simple one–way ANOVA. Multiple comparisons were performed by Tukey's method.

Results

Salt effects in the birch soil

Methane oxidation approximated an exponential decline in all soil samples (Fig. 7), although rate constants varied among treatments (Fig. 8). CH_4 oxidation kinetics with deionized water and all $SO_4^=$ salts were clearly first–order ($r^2>0.995$ in all cases; Fig. 8). Neither K_2SO_4 nor Na_2SO_4 significantly inhibited CH_4 oxidation compared with deionized water ($p=0.78$), and the curves for K_2SO_4 and Na_2SO_4 were nearly identical ($p=0.91$; Na_2SO_4 data not shown). $(NH_4)_2SO_4$ inhibited CH_4 oxidation by approximately 14% relative to K_2SO_4 ($p<0.01$), and 19% relative to deionized water. Inhibition did not vary with CH_4 concentration.

At the higher salt concentration (5.6 μmol•(g dry soil)$^{-1}$), KCl and NH_4Cl both inhibited CH_4 oxidation severely (81.6% ± 1.1, on average), and their effects were indistinguishable ($p=0.84$). Slopes of oxidation rate vs. CH_4 concentration were significantly different from zero ($p<0.01$), but did not fit a first–order model as tightly as did the $SO_4^=$ salts (Fig. 8). The lower Cl^- concentration (0.85 μmol•(g dry soil)$^{-1}$) also inhibited CH_4 oxidation severely (76–88%; Fig. 9). At this concentration, the degree of inhibition increased as CH_4 concentration increased ($p<0.001$), but there was no difference between K^+ and NH_4^+ ($p=0.89$).

Water potential was -0.15 MPa in the deionized water controls, and -0.18 MPa in the salt treatments, on average. The salt treatments were not significantly different from the water controls.

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Salt effects in other soils

The other three soils examined yielded three distinct responses to salt additions (Fig. 10). The white spruce site was highly sensitive to salt in general. CH_4 oxidation was inhibited by both $SO_4^=$ (39% inhibition; $p<0.001$) and Cl^- salts (78% inhibition, $p<0.001$), but no specific NH_4^+ effect was evident (NH_4^+ vs K^+: $p=0.95$). In contrast, the shrub tundra soil showed no sensitivity to either K_2SO_4 ($p=0.91$) or KCl ($p=0.82$), yet was sensitive to NH_4^+ in general (22% inhibition; $p=0.015$). The wetland taiga soil exhibited no sensitivity to any salt ($p=0.83$). CH_4 oxidation rates also varied among these soils. The control rates were 55.4, 105.1, and 1085 pmol $CH_4 \bullet (g\ dry\ soil)^{-1} \bullet h^{-1}$ for the spruce taiga, shrub tundra, and wetland taiga sites, respectively.

Discussion

Mechanism of Salt Effects

Osmotic effects. In all treatments, water potentials were well within the range generally considered ample for microbial activity (22, 40, 59). The mean water potential in the salt treatments (-0.18 MPa) was near the upper end of the optimum range (-0.35 to -0.15) determined for three taiga soils by manipulating soil water content (21). It is not likely, therefore, that osmolarity significantly inhibited CH_4 oxidation in this experiment, although it is interesting that the mean difference in water potential between the deionized water controls and the K_2SO_4–treated samples (6.7%) was similar to the (nonsignificant) difference between the corresponding rate constants for CH_4 oxidation (5.1%). Nesbitt & Breitenbeck (37) concluded that osmolarity diminished CH_4 uptake in a salt–amended Louisiana soil, but this was with a salt concentration (35 μmol·g $soil^{-1}$) considerably higher than those used in the present study. Thus, we conclude that salts can cause osmotic stress at higher concentrations but that inhibition of CH_4 oxidation in this study resulted primarily from specific ion toxicities.

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Specific ion toxicities. Inhibition due to any ion may result either from a general ionic effect, or from specific toxicity. In order to discern a specific effect it is necessary to demonstrate that a similar ion does not duplicate the effect. In this experiment, we used K^+ as a control for nonspecific NH_4^+ effects. One could argue that K^+ may inhibit CH_4 oxidation by a different mechanism than NH_4^+. There is no evidence, however, to support this idea. K^+ is often the most abundant intracellular solute in bacteria, and is unlikely to inhibit specific cellular processes except at concentrations high enough to induce osmotic effects (31). Thus, it is reasonable to assume that any K^+ effect would be nonspecific, and that similar cations would induce the same effect. K^+closely resembles NH_4^+ physically (more so than Na^+; Table 3), and has similar chemical attributes in soil (51) and inside bacterial cells (31). We conclude, therefore, that K^+ is an appropriate control ion for nonspecific NH_4^+ effects.

It is also important to use an appropriate counterion. In the birch soil, NH_4Cl and KCl inhibited CH_4 oxidation to the same extent, thus revealing no specific NH_4^+ effect (Fig. 8, 9). Yet, comparing the effects of $(NH_4)_2SO_4$ and K_2SO_4 revealed NH_4^+–inhibition of 14% (Fig. 8). Thus, although CH_4 oxidation was sensitive to NH_4^+, the extreme Cl^- toxicity masked this fact. Had $SO_4^=$ salts not been examined, no NH_4^+–inhibition would have been observed. Alternatively, if NH_4Cl had been examined without the KCl control, the Cl^-–inhibition of >80% could have been mistaken for NH_4^+–inhibition. Because sensitivities vary, counterions should be selected carefully for each soil. Whalen & Reeburgh (55) used NH_3 gas to avoid potential salt effects. Although this approach also has potential problems, such as altering soil pH (54), it may provide a viable alternative for soils that are particularly sensitive to salts.

The four soils examined exhibited four distinct ion–sensitivity patterns (Fig. 9, 4). In both of the upland taiga soils CH_4 oxidation was severely inhibited by Cl^-, which was much more toxic than $SO_4^=$. The same relationship was observed

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in a Colorado grassland soil (7), suggesting that Cl^- toxicity to soil CH_4 oxidizers may be common. Other soil microbial processes have also exhibited greater sensitivity to Cl^- than to $SO_4^=$ (14, 47). In contrast, the shrub tundra and wetland taiga soils showed no sensitivity to either Cl^- or $SO_4^=$. Because the two organic–rich soils had higher absolute water contents than the two upland taiga mineral soils, the salt concentration was comparable with that in the low–salt–concentration experiment in the birch soil, which showed strong inhibition. The higher cation exchange capacities in the organic soils, however, would reduce the activity of the ions, and therefore their effects on microorganisms. Hence, it is possible that the lack of inhibition in the organic soils was due to less salt–sensitive CH_4 oxidizers than in the mineral soils, but lower ion activities could also explain the results.

The diversity of biological responses to similar salt treatments suggests that the organisms differ physiologically among the soils. This is particularly true when comparing the birch and spruce taiga soils. Since these soils are physically and chemically similar, the different ion sensitivities likely resulted from distinct biological responses to the same chemical cues. These results support the hypothesis previously put forth by Gulledge et al. (20) that the variability of NH_4^+–inhibition dynamics reported in the literature results from the distribution of physiologically distinct CH_4 oxidizer populations across sites, and that this is the primary landscape–level control on NH_4^+–inhibition of soil CH_4 consumption.

Implications for experimental design

The lack of salt controls can lead to misinterpretation of salt–related inhibition. Several studies examining the effects of multiple salts have reported a variety of inhibition responses. Crill et al. (10) and Kightley et al. (26) noted that much of the inhibition observed in their respective studies could be accounted for by the effects of ions other than NH_4^+. There are published studies in which the lack of a salt control may have led to erroneous conclusions, or where employing a salt control

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might have facilitated interpretation of results. For example, the mechanism of NH_4^+–inhibition was investigated in temperate forest soils by comparing the effect of NH_4Cl with deionized water controls at various CH_4 concentrations (29, 45). The authors determined that the degree of inhibition increased with CH_4 supply, and interpreted this phenomenon as NH_4^+–inhibition resulting from the production of NO_2^-, a toxic intermediate of NH_4^+ oxidation. In our low–concentration NH_4Cl treatment, we also observed this pattern of increasing inhibition (Fig. 9). Since equivalent KCl additions produced identical results, however, the inhibition dynamics did not require NH_4^+, and could not have involved NO_2^-. Similar results were observed in a Louisiana soil, in which KCl–inhibition was greater at 100 ppm CH_4 than at 3 ppm (37). Thus, Cl^- toxicity, rather than NH_4^+–inhibition, was the most probable mechanism of inhibition. Why Cl^- toxicity increased with CH_4 supply is unclear; perhaps increased metabolic activity accelerated Cl^- transport into cells. Given our experience with Cl^-, and salt amendments in general, the NO_2^-–inhibition hypothesis (29, 45) requires evidence that the observed inhibition dynamics are unique to NH_4^+, and cannot be duplicated by K^+ salts. Otherwise, Cl^- toxicity should be considered a likely mechanism.

Although it is particularly important to consider salt and ion effects in short–term laboratory studies, they may also explain some of the observed inhibition of CH_4 consumption *in situ*. Field studies typically examine longer–term fertilization effects than do laboratory incubations, and salt effects have usually been ignored or assumed to be unimportant since salts would be leached out of the system. Field experiments, however, often involve high levels of chronic fertilization, which could elevate soil ion concentrations for extended periods of time. In some cases, lingering Cl^- or $SO_4^=$ ions may explain the persistence of inhibition even after NH_4^+ concentrations have declined (e. g., 24, 37, 45). It seems less likely that NO_3^- ions would persist, but frequent NH_4NO_3 application could elevate soil ion concentrations, depending on the nature of the system and the

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amount of fertilizer added. In field studies using urea (e. g., 35), long–term effects are likely due to NH_4^+–inhibition (assuming no change in soil pH), since urea hydrolysis does not produce other ions.

In situ CH_4 consumption in agricultural soils in Germany and England was strongly inhibited by inorganic NH_4^+ fertilizers (NH_4NO_3 or $(NH_4)_2SO_4$), but not by farmyard manure amendments producing similar soil NH_4^+ concentrations (23, 58). When NH_4NO_3 was added chronically to manure plots, however, oxidation rates declined (58). The authors interpreted this result as a decline in methanotroph populations due to intensified resource competition from nitrifiers. Although this hypothesis is plausible, elevated inorganic ion concentrations could produce the same results. Parallel application of KNO_3 to manure plots could have helped determine whether ion effects were involved.

Implications for the impact of NH_4^+ on the soil CH_4 sink

The degree of NH_4^+–inhibition reported here (14% for birch; 22% for shrub tundra) is small compared with that reported in several other studies, despite our relatively large NH_4^+ additions. We believe this is because we corrected for salt effects when calculating specific NH_4^+–inhibition. A study in a Colorado prairie soil reported 73% inhibition due to NH_4NO_3 additions (7). Equivalent KNO_3 additions, however, induced 46% inhibition. Thus, NH_4^+ can only be said to have specifically inhibited CH_4 oxidation by 27%, a figure similar to the levels reported here. Several studies have reported severe NH_4^+–inhibition (>50%) without the benefit of a salt control (e. g., 3, 45, 2), and may therefore have over–estimated the specific NH_4^+ effect.

Many factors other than fertilization may reduce soil CH_4 uptake, including a variety of agrochemicals (7, 52), sawdust amendments (44), physical disturbances (39), changes in soil moisture, pH, and organic matter content (1, 12, 16, 17, 24, 48, 56), and air pollution (19). Thus, apparent NH_4^+ effects may be the combined

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result of several factors that coincide with fertilization. Clearly, NH_4^+ inhibits CH_4 uptake in some soils, but perhaps NH_4^+–inhibition has been over–emphasized at the expense of other equally important, disturbance–related controls on soil CH_4 consumption.

There are two major conclusions from this work. First, by not distinguishing between specific NH_4^+ effects and NH_4^+–salt effects, some studies may have misinterpreted the mechanism of NH_4^+–inhibition of soil CH_4 oxidation. Second, the impact of NH_4^+, *per se*, on the soil CH_4 sink may previously have been overestimated. To avoid these potential pitfalls, future field and laboratory studies should incorporate salt controls, and researchers should carefully consider whether salt additions are appropriate for a particular soil.

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Table 2. Soil properties and site locations

soil type	soil texture	% organic matter (±S. E.)	water holding capacity ($g\ H_2O \bullet g\ dry\ soil^{-1}$)
upland birch[a]	fine silt	3.68(0.06)	0.62
white spruce[b]	fine silt	4.42(0.10)	0.66
shrub tundra[c]	silt loam	n.d.	2.50
taiga wetland[b]	coarse, fibrous	61.0(0.6)	4.70

[a]University of Alaska Arboretum, Fairbanks

[b]Bonanza Creek Experimental Forest, west ridge of the Tanana River valley, Alaska

[c]Toolik Lake area, foothills of the Brooks Range north slope, Alaska

Table 3. Physical properties of NH_4^+, K^+, and Na^+

ion	hydration energy (38) (DH; $kcal \bullet mol^{-1}$)	hydrated radius (4) (nm)	crystal radius (4) (nm)
NH_4^+	-73	0.331	0.148
K^+	-75	0.331	0.133
Na^+	-95	0.358	0.095

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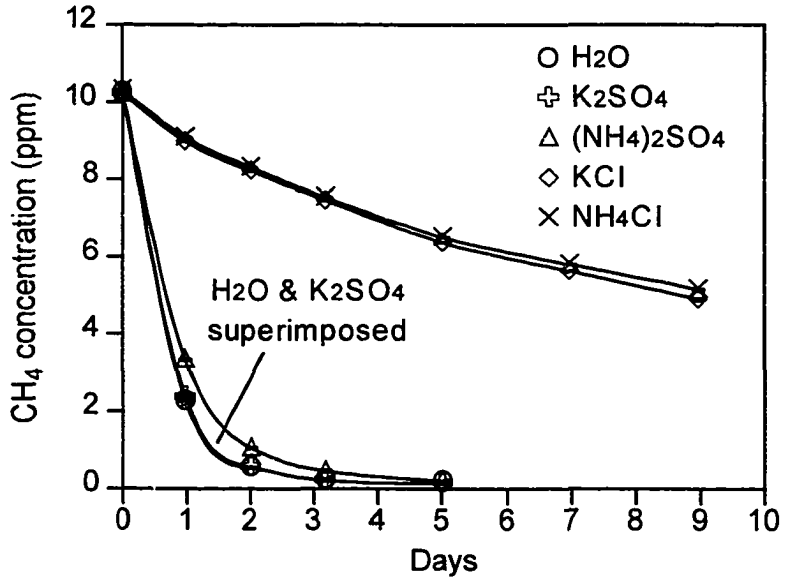

Figure 7. Typical CH_4 oxidation curves for salt treatments in the birch taiga soil. Error bars (± S. E.) are contained within the symbols.

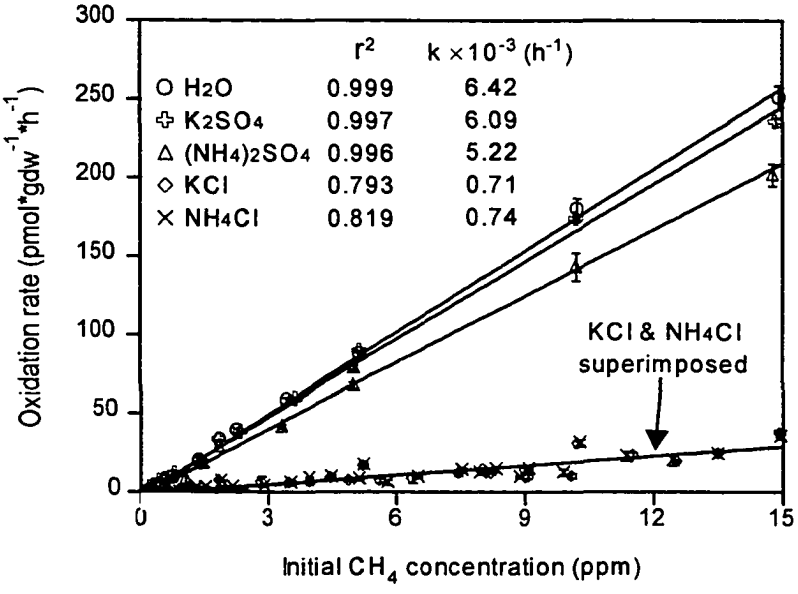

Figure 8. CH_4 oxidation kinetics in response to salt treatments in the birch taiga soil. The linear correlation coefficient (r^2) and rate constant (k) are tabulated. When error bars (± S. E.) are not visible, they are contained within the symbols.

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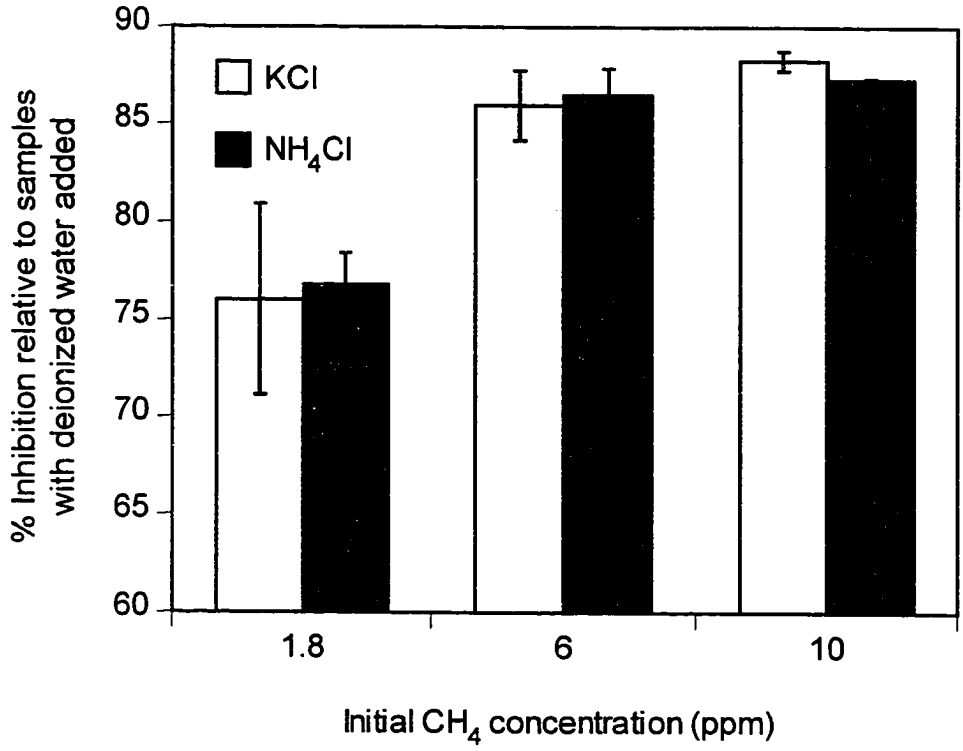

Figure 9. Effect of CH_4 concentration on relative inhibition of oxidation rates by KCl and NH_4Cl (0.85 μmol•gdw^{-1}) in birch soil. Error bars represent ± S. E.

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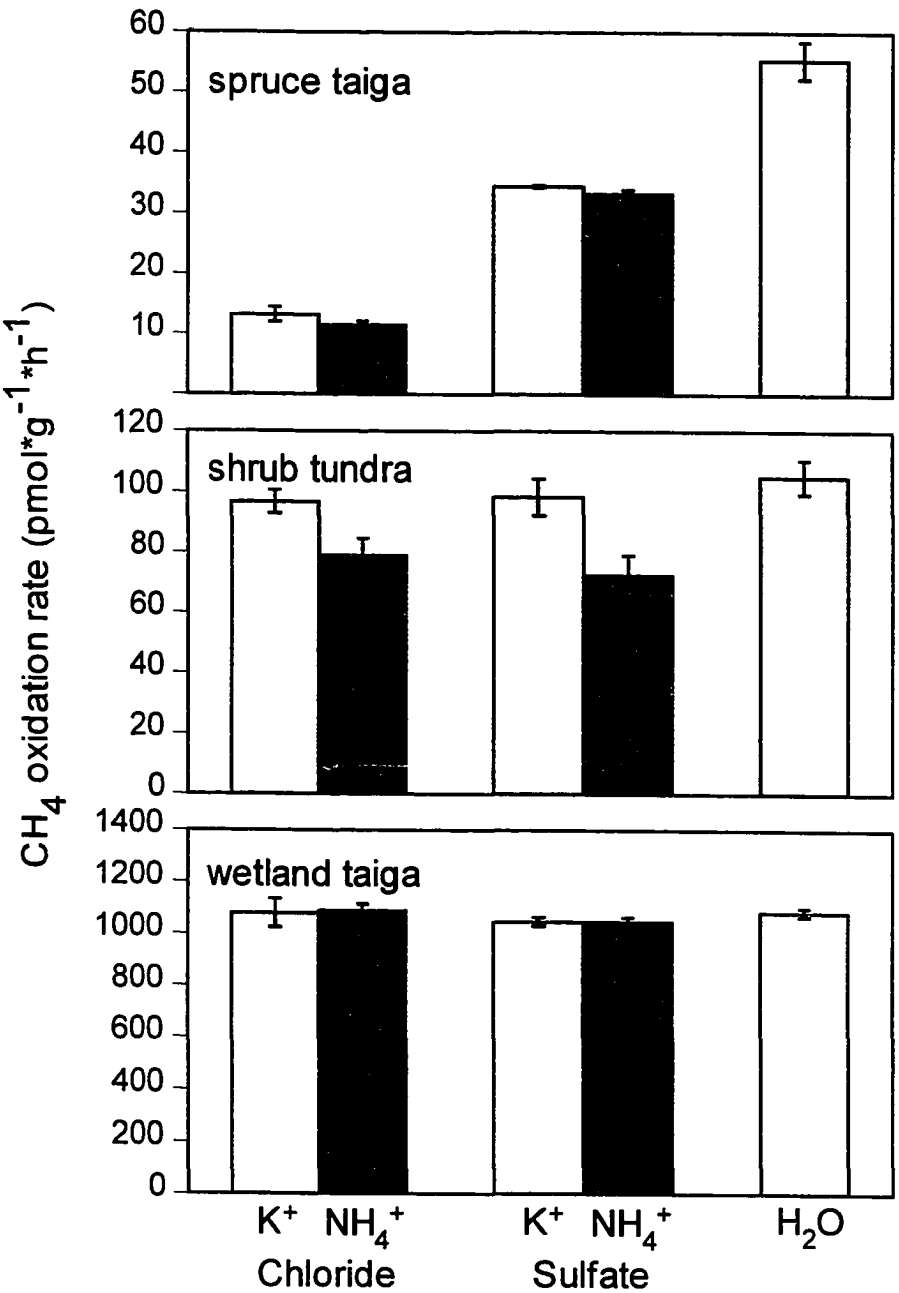

Figure 10. Salt effects on CH_4 oxidation in spruce taiga, shrub tundra, and wetland taiga soils at an initial CH_4 concentration of 10 ppm. Error bars represent ± S. E.

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V. Different NH_4^+–Inhibition Patterns of Soil CH_4 Consumption: A Result of Distinct CH_4 Oxidizer Populations Across Sites?†

Introduction

Biological CH_4 consumption directly from the atmosphere in well–drained soils is estimated to comprise 3% to 9% of the global atmospheric CH_4 sink (Houghton et al. 1995) and plays a key role in global CH_4 cycling (Reeburgh et al. 1993). The vagueness of available estimates is exacerbated by a poor understanding of how CH_4 oxidation is controlled at the ecosystem level. NH_4^+–fertilization inhibits CH_4 consumption in a variety of ecosystems and represents a means by which human activity could reduce the atmospheric CH_4 sink (Keller et al. 1990; King 1992; Ojima et al. 1993; Schimel et al. 1993). NH_4^+–inhibition in the field was first reported by Steudler et al. (1989) in several temperate forest sites. Since then there have been reports of inhibition in temperate and taiga forests (Whalen et al. 1991; Adamsen & King 1993; Schnell & King 1994; Castro et al. 1995), grasslands (Mosier et al. 1991), alpine tundra (Neff et al. 1994), agricultural soils (Conrad & Rothfus 1991; Hütsch et al. 1993), and aquatic sediments (King 1990; Conrad & Rothfus 1991; Bosse et al. 1993). As land–use changes and N deposition increase globally, NH_4^+–inhibition becomes an increasingly important factor in the global CH_4 cycle (Ojima et al. 1993).

The direct physiological cause of inhibition is unclear and may vary (Dunfield & Knowles 1995). Methanotrophic bacteria, thought to consume atmospheric methane in most soils (King 1992; Bender & Conrad 1994), oxidize methane via monooxygenase enzymes (Holmes et al. 1995). Physical similarities between CH_4 and NH_3 permit both compounds to compete for binding sites on this enzyme. Thus, NH_3 oxidation can competitively inhibit CH_4 oxidation (Bédard & Knowles

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†J. Gulledge, A.P. Doyle & J.P. Schimel. Submitted to *Soil Biology & Biochemistry*

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1989; Carlsen et al. 1991; Dunfield & Knowles 1995). Another inhibition mechanism that has been demonstrated is toxicity of NO_2^-, which can be produced from NH_3 oxidation (King & Schnell 1994a,b; Schnell & King 1994; Dunfield & Knowles 1995).

In the field, inhibition dynamics vary. In some cases the CH_4 consumption rates decrease immediately (within hours or days) following fertilization (Adamsen & King 1993; Crill et al. 1994; Schnell & King 1994). This short–term inhibition could be explained by substrate competition, NO_2^- toxicity, or both (Schnell & King 1994; Dunfield & Knowles 1995). Other inhibition patterns, however, have also been observed. Inhibition may be delayed, developing over several years, or may never occur despite repeated annual fertilization (this study; Whalen et al. 1991; Hütsch et al. 1993). Such responses are difficult to explain by direct enzymatic inhibition or NO_2^- toxicity, both of which should evoke an immediate response (assuming the CH_4–oxidizing organisms are actually exposed to NH_4^+). More investigation is needed to explain the dynamics of CH_4 oxidation inhibition in soils exhibiting either delayed inhibition or no inhibition.

We studied two upland forest sites in the Alaskan taiga that display non–immediate responses. In a paper birch (*Betula papyrifera*) stand, no inhibition was apparent during a single–season fertilization experiment, but was severe in plots that had been fertilized for 3 or more field seasons (Fig. 11). These results indicated that the onset of NH_4^+–inhibition of soil CH_4 consumption was delayed in this birch stand. The other site, a white spruce (*Picea glauca*) stand, also was fertilized for 5 consecutive years, yet no inhibition developed (data not shown). We hypothesized that such dynamics could be expected in soils where added NH_4^+ is quickly immobilized or nitrified, thereby protecting CH_4 oxidizers from exposure to the inhibitory compound. Delayed inhibition development could then be due to initially rapid NH_4^+ immobilization in early years, followed by N-saturation, and concomitant exposure of CH_4 oxidizers to NH_4^+. Sites exhibiting no inhibition

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would then result from increased capacity to buffer against N–saturation. Alternatively, one or more novel physiological mechanisms of inhibition could be involved. Methane oxidizers in sites that fail to exhibit inhibition could simply be insensitive to NH_4^+. The objective of this study was to test these hypotheses by determining the basis of the different fertilization responses of soil CH_4 consumers in the birch and spruce sites.

Materials and Methods

We monitored the effects of annual fertilization on CH_4 consumption in the taiga forest sites for 5 consecutive snow–free field seasons (approximately early June to early September). The general approach was to facilitate meaningful interpretation of the results of the field experiments by using controlled laboratory manipulations of soil samples from field sites.

Field sites

The two study sites, one paper birch stand and one white spruce stand, are located in the Bonanza Creek Experimental Forest (64°45'N, 148°18'W) approximately 25 km southwest of Fairbanks, Alaska. These sites were established in 1987 as part of the U.S. Long–Term Ecological Research program (LTER). As such, a large body of ancillary data, including CH_4 fluxes, is collected throughout each snow–free season. Both sites are on south–facing slopes situated on the north ridge of the Tanana River valley at approximately 430 m elevation. Both are well–drained and underlain by a silty, micaceous loess. These sites represent distinct stages of a post–fire successional sequence. The birch and spruce sites are approximately 80 and 200 years post–burn, respectively.

The birch–dominated site also contains white spruce, quaking aspen (*Populus tremuloides*), and balsam poplar (*Populus balsamifera*). The understory is sparse, consisting largely of *Rosa acicularis*, *Viburnum edule*, *Salix* spp., and *Equisetum*

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spp. The O–horizon results primarily from decomposition of birch litter and fine roots. The forest floor is 5–10 cm thick and the transition to mineral soil is abrupt. The spruce site is almost pure white spruce with occasional patches of alder (*Alnus crispa*) in the understory. The forest floor has a near–continuous cover of feather moss, predominantly *Hylocomium splendens*, which tends to retain water and insulate the underlying mineral soil from temperature and moisture fluctuations. The 15–20 cm thick O–horizon consists primarily of dead moss, and the transition to mineral soil is abrupt. Detailed descriptions of these and similar sites are provided by Viereck et al. (1986) and Van Cleve et al. (1991).

Field experiments

In May 1990, paired 15m × 15m plots were established in each site (primary plots). Fertilizer (NH_4NO_3) was applied to one plot (fertilized) in each site by dry broadcast each June. The applications were intended to double net annual N mineralization in each site (Van Cleve, pers. comm.). The birch and spruce sites received 60 and 50 kg $N \cdot ha^{-1} \cdot yr^{-1}$, respectively. The second plot served as a control. Triplicate static–flux chambers were permanently deployed in each plot. CH_4 fluxes were measured every two weeks throughout the snow–free season as described by Whalen & Reeburgh (1988). Flux measurements began in 1991 in the birch site and 1993 in the spruce site.

Because flux measurements did not begin in the first season of fertilization, a second set of plots (secondary plots) was established in both sites in June 1993, in order to examine the short–term response of soil CH_4 consumption to fertilization. Triplicate 2.25–m^2 plots were treated uniformly with 4 liters of 1.1 M NH_4Cl (62 kg $N \cdot ha^{-1}$). Paired control plots received 4 liters of deionized water, equivalent to 0.17 cm precipitation. One static–flux chamber was then deployed in each plot. Another 4 liters of deionized water was applied to each plot three days later to

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enhance fertilizer penetration into the soil. The first flux measurements were taken 5 days later to allow soil moisture to re–equilibrate with ambient conditions.

Laboratory experiments

Triplicate cores (to 40 cm mineral soil depth) were extracted from each primary control and fertilized plot in the birch and spruce sites. The organic soil was removed from each core and the mineral soil was sectioned in the field into four 10–cm depth intervals. The samples were placed in perforated plastic bags to avoid CH_4 and O_2 depletion and stored in the laboratory overnight. Sample processing began within 16 hours of collection. Each core section was homogenized by sieving through a 4 mm screen. Water holding capacity was determined gravimetrically for each depth interval and soil moisture was adjusted so that the final water content after substrate addition would be 30% of water holding capacity, a previously determined optimum for CH_4 oxidation (Chapter III). Each core section was then subsampled into triplicate 235 ml Mason jars and treated with either deionized water (control), K_2SO_4 solution (salt control), or $(NH_4)_2SO_4$ solution (NH_4^+–fertilizer treatment). The $(NH_4)_2SO_4$ treatment was designed to add 75 μg NH_4^+–N•g dry soil^{-1}; K_2SO_4 addition was equimolar with $(NH_4)_2SO_4$. K^+ and $SO_4^=$ were chosen as control ions because they are relatively nontoxic to soil organisms, particularly in comparison with Na^+ and Cl^- (Chapter IV; Killham & Firestone 1984; Stark & Firestone 1995). Moisture was allowed to equilibrate overnight. Preparation between field collection and initiation of the experiment required 1 week. Soil samples were continuously exposed to laboratory air throughout this period to prevent CH_4 and O_2 depletion. Subsampling the triplicate cores yielded triplicate samples for each laboratory treatment at each core depth for both control and fertilized plots.

On the first day of the experiment, the headspace of each jar was equilibrated with laboratory air (~1.8 ppm CH_4) and the jars were sealed using lids fitted with

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butyl rubber septa to allow headspace sampling. The jars were then incubated overnight (~16 h). CH_4 consumption rates were determined by difference in CH_4 concentration in the headspace at the beginning and end of the assay period. We chose a two–point rate calculation because sample loads were large, and previous work indicated that CH_4 oxidation rates were linear with respect to CH_4 concentration (first–order) over the course of the incubation period. CH_4 concentration was measured on a Shimadzu GC–14A gas chromatograph equipped with a flame ionization detector and a 2–m stainless–steel column (3.2-mm i.d.) hand–packed with Poropak Q (Supelco, Bellefonte PA). The procedure was repeated on the 10th day of the experiment to determine whether fertilization effects on CH_4 consumption had changed over this period. In the meantime, the lids were inverted and loosely fitted on the jars to allow gas diffusion between the headspace and laboratory air. This procedure was to supply constant atmospheric CH_4 and O_2 to the soil while minimizing advective water loss. Thus, any water loss should be due to diffusion and should be similar in all jars. Lost moisture was not replaced due to the potentially deleterious effects of rewetting on microbial populations (Kieft et al. 1987; Clein & Schimel 1993; Van Gestal et al. 1993). Water loss was not determined for each jar, but random sampling indicated that it was $<10\%$.

Net N mineralization was also measured over the 10–day incubation period. On day 1 subsamples of each core section (with and without $(NH_4)_2SO_4$ added) were extracted with 2N KCl solution. On day 10, the soil samples in the jars were also extracted in this manner. NH_4^+ and NO_3^- concentrations were measured on a Lachat autoanalyzer using standard methods (Keeney & Nelson 1982). Net mineralization was calculated as the change in extractable inorganic N over 10 days.

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Statistical analyses

Field data were analyzed by 2–way, repeated–measures ANOVA with sample date and treatment as factors. Laboratory data were analyzed by simple 2–way ANOVA using salt treatment and soil depth as factors. Tukey multiple comparisons were used to detect differences among treatment groups.

Results

Field study

In the birch site, inhibition was not obvious in the first year of fertilization as determined from the secondary plots ($p=0.45$). Although NH_4Cl was used in the secondary plots, the lack of inhibition nullifies the issue of specific Cl^- toxicity. In the primary plots, however, relative inhibition was 49% in year 2, and reached 60–70% in subsequent years (Fig. 11; $p<0.001$). Inhibition never occurred in the spruce site ($p=0.90$; results not shown). For the purposes of the present study, other field data are irrelevant. A detailed description of the field study, which also examined CO_2 fluxes, is in preparation.

Laboratory study

In the control birch soil and both the control and fertilized spruce soils, oxidation capacity decreased with depth (Fig. 12a,b). CH_4 oxidation rates on day 1 in field–fertilized soil from the birch site were depressed 40 to 70% in the upper 20 cm mineral soil relative to the control soil (Fig. 12a; $p=0.05$). These data are consistent with the field inhibition observed in the birch site. There was no fertilization effect below 20 cm. In the spruce site, there was no difference in oxidation rates between control and fertilized soils (Fig. 12b; $p=0.45$), which is also consistent with the absence of *in situ* inhibition in this site. The maximum CH_4 oxidation rates were almost an order of magnitude higher in the spruce soil than in the birch soil (Fig. 12a,b).

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Adding dissolved NH_4^+ salt directly to homogenized soil samples ensured exposure of CH_4 oxidizers to NH_4^+, unless the soil's immobilization capacity was great enough to protect them. The effects of adding H_2O, $(NH_4)_2SO_4$, or K_2SO_4 on CH_4 oxidation are depicted in Fig. 13. There was no apparent inhibition due to any treatment in either the control or fertilized birch soil (Fig. 13a). In the spruce soil, CH_4 oxidation was sensitive to both the K^+ and NH_4^+ salts relative to the control ($p<0.001$), but there was no NH_4^+–inhibition relative to K^+ ($p=0.68$; Fig. 13b). In no case (field or laboratory), then, were we able to induce short–term inhibition in either the birch or the spruce soil due to a selective NH_4^+ effect.

In the birch soil incubated for 10 days after NH_4^+ addition, CH_4 oxidation capacities remained unchanged in the 0 to 20 cm samples (Fig. 14). In the 20 to 40 cm birch soil, however, oxidation capacity increased up to 5-fold in the presence of H_2O or K_2SO_4 ($p<0.001$), bringing the activity in the deeper soils up to levels similar to those in the shallower soils (Fig. 14a,b). Adding $(NH_4)_2SO_4$ prevented this increase in oxidation capacity as there was no significant difference between oxidation rates on day 1 and day 10 in the lab–fertilized samples (Fig. 14c; $p=0.36$). In the spruce soil, there were no changes in CH_4 oxidation rates under any circumstances ($p=0.45$; data not shown).

Initial extractable NH_4^+ concentrations were low in all cases, but significantly higher ($p<0.001$) in the spruce site (0.6 to 6.0 ppm) than in the birch site (0.02 to 0.6 ppm). When no NH_4^+ was added, the spruce soil mineralized N faster on average than the birch soil ($p<0.05$; Fig. 15a). When $(NH_4)_2SO_4$ was added, the birch site exhibited net N immobilization rates slightly higher than the spruce site ($p<0.05$; Fig. 15b). On average, only about one–third of the NH_4^+ initially present was immobilized over the 10–day assay period. In no case was all the added NH_4^+ immobilized.

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Discussion

Recent work in this and other laboratories has revealed multiple inhibition patterns of soil CH_4 consumption following NH_4^+–fertilization. These patterns range from immediate (within hours to days) inhibition to delayed (months to years) inhibition to complete absence (over several years) of inhibition. Each of these ecologically distinct inhibition patterns could result from the same physiological mechanism(s), with the soil properties of each system differentially regulating exposure of the CH_4 oxidizers to NH_4^+, or from physiologically distinct responses of the methane oxidizers to NH_4^+.

Ecological Basis of Inhibition

Short–term inhibition has been observed in field and laboratory investigations and in a diverse array of ecosystems. CH_4 oxidation was inhibited in temperate pine–hardwood forest soils within two hours of adding small amounts (1 μmol•g soil^{-1}) of NH_4^+ to intact soil cores (Adamsen & King 1993). In similar soils, approximately 30% inhibition of CH_4 consumption occurred in field plots within 2 days of fertilization, and in laboratory samples within one hour of adding only 1 μmol N•g^{-1} soil (Schnell & King 1994). CH_4 consumption in northern peat soils was inhibited within 3 weeks after fertilization, both in the field and in intact soil cores (Crill et al. 1994). A Louisiana wetland soil exhibited inhibition in laboratory assays within 3 days after NH_4^+ addition (Nesbit & Breitenbeck 1992). Short–term NH_4^+–inhibition also occurred in slurries of an agricultural humisol (Dunfield & Knowles 1995), and in microcosms of upland taiga soils amended with NH_3 gas (Whalen & Reeburgh, pers. comm.).

Short–term inhibition of soil CH_4 consumption requires a physiological mechanism by which NH_3/NH_4^+ directly decreases the enzymatic efficiency of CH_4 turnover, or by which the concentration of CH_4–oxidizing enzymes in the soil is suddenly decreased (*e.g.*, by cell death). In pure cultures of methanotrophic

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bacteria, NH_3 can competitively inhibit CH_4 oxidation (Carlsen et al. 1991). Dunfield & Knowles (1995) demonstrated that simple competition accounted for inhibition of CH_4 oxidation in an agricultural humisol. Another mechanism capable of immediate inhibition is acute chemical toxicity by NO_2^- or NH_2OH produced from NH_3 oxidation. (King & Schnell 1994a,b; Schnell & King 1994; Dunfield & Knowles 1995). Kightley et al. (1995) also suggested that immediate inhibition may at times result simply from nonspecific ionic effects due to salt additions, rather than from a selective NH_4^+ effect. Immediate inhibition is common and can be explained by any of these physiological mechanisms. Because immediate inhibition is conspicuous, its ramifications and mechanism(s) have received much attention from researchers.

Immediate inhibition (by any physiological mechanism) is, however, only one ecological response to N fertilization in the field. Two other patterns have also been observed: no inhibition and delayed inhibition. In our spruce site, 5 years of annual fertilization failed to inhibit field flux rates. Laboratory assays also failed to induce inhibition. Whalen & Reeburgh (1991) observed a similar lack of inhibition in a taiga forest fertilized each summer month for 2 years. Lack of NH_4^+–inhibition was also observed in two fertilized agricultural soils (Bronson & Mosier 1993; Dunfield et al. 1995), although the studies were of shorter duration than the taiga studies. Under no circumstances did NH_4^+ perceptibly suppress CH_4 oxidation in our white spruce site. These results suggest either that the CH_4 oxidizers in this soil are insensitive to NH_4^+, or that other soil properties protect the CH_4 oxidizers from exposure to NH_4^+.

Our birch site provides an example of delayed inhibition. We were unable to induce an NH_4^+ effect within 10 days of adding $(NH_4)_2SO_4$ directly to homogenized birch soil samples, despite the fact that inhibition in the field had become severe (60–70%) by the third year of fertilization. Similarly, Hütsch et al. (1993) reported that long-term fertilization regimes had substantially suppressed field CH_4

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consumption rates (50–80% inhibition) in temperate arable soils, yet the short–term effects of adding NH_4^+ in the laboratory were negligible over a 9 day assay period. These results suggest that inhibition development in the field was delayed relative to the time of initial fertilization. One could argue that the NH_4^+ concentrations in our laboratory study were too low to induce inhibition, but we used concentrations at least 5 times those used by Adamsen & King (1993), Schnell & King (1994), and Dunfield & Knowles (1995), all of whom observed immediate inhibition. Thus, we believe our results truly indicate a lack of short–term NH_4^+–inhibition.

What is the ecological cause of delayed inhibition? We suggest two possibilities: soil N dynamics could protect the CH_4 oxidizers from exposure to NH_4^+, or the physiological inhibition mechanism(s) could be different from those that cause immediate inhibition. We originally hypothesized that the inhibition dynamics in our taiga sites could have resulted from NH_4^+ immobilization or nitrification that initially buffered the methane oxidizers from exposure to NH_4^+. Eventually, however, continued fertilization caused the birch soil to become N–saturated and the CH_4 oxidizers became exposed to NH_4^+. This scenario would result in delayed inhibition, as observed in the birch site. The spruce site, by this hypothesis, had not yet become N–saturated, presumably due to enhanced immobilization or nitrification relative to the birch soil. Continued NH_4^+–fertilization would eventually saturate this soil, as well, and induce inhibition. This hypothesis has two testable implications: 1) that large additions of NH_4^+ directly to the mineral soil would overcome immobilization and cause immediate inhibition, and 2) that the spruce soil would have a significantly greater immobilization–nitrification capacity than the birch soil in order to protect the methane oxidizers from NH_4^+ even after 5 years of fertilization. Neither of these predictions held.

As previously discussed, we could not induce short–term inhibition of CH_4 oxidation in the birch soil (Fig. 13), despite large NH_4^+ additions. This result

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falsifies the first prediction. In the laboratory study, the spruce soil had greater extractable NH_4^+ concentrations and net mineralization rates than those in the birch soils (Fig. 15). These data suggest that, if anything, CH_4 oxidizers in the spruce soil were more likely to be exposed to NH_4^+ than those in the birch soil. Field estimates of annual net N mineralization in nearby birch and spruce plots were similar for the two sites (60 and 50 kg $N \cdot ha^{-1} \cdot yr^{-1}$, respectively; Van Cleve, pers. comm.). Additionally, nitrification potentials were very low in both sites (unpublished data) suggesting that nitrifiers played an insignificant role in consuming NH_4^+. The lack of distinction in the native N dynamics of the two sites, combined with the small magnitudes of immobilization relative to the amount of NH_4^+ added in the laboratory (Fig. 15b), invalidates the second prediction. We found no support for the hypothesis that N–cycling dynamics in the two sites explained either the delayed inhibition in the birch site or the lack of inhibition in the spruce site. We conclude, therefore, that delayed inhibition in the birch site resulted from a different physiological mechanism than those that induce short–term inhibition (substrate competition, NO_2^- toxicity, or general ion toxicity). We also conclude that the spruce site has a population of CH_4 oxidizers that is insensitive to NH_4^+. Thus, the different responses to fertilization between the birch and spruce sites (delayed vs. no inhibition) were likely due to the occurrence of physiologically distinct CH_4 oxidizer populations.

Possible Mechanism of Delayed Inhibition

Delayed inhibition, as in our birch site and the arable soils of Hütsch et al. (1993), defies explanation by enzyme competition or acute toxicity. Although the actual mechanism requires further investigation, our results suggest that repression of CH_4 oxidizer growth provides a viable explanation of the inhibition dynamics in the birch site. Soil sampled from 20–40 cm mineral soil depth had a lower CH_4 oxidation capacity initially than shallower soils (Fig. 12a). This was not surprising

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since most of the CH_4 diffusing into the soil is oxidized in the top 20–30 cm (Whalen et al. 1991, 1992). Therefore, the CH_4 oxidizer population should decrease with depth below 20 cm, assuming they rely on CH_4 for survival. During the 10–day incubation with ambient atmospheric CH_4 (1.8 ppm), however, oxidation capacities in the deeper birch soil increased 2– to 5–fold relative to their rates on day 1 (Fig. 14a,b). This response represents a positive physiological change in the CH_4 oxidizer population and probably indicates *de novo* enzyme synthesis, and is also consistent with a population increase. Since this response probably resulted from exposing CH_4 oxidizers that typically experience less than 0.5 ppm CH_4 to atmospheric CH_4, this physiological change suggests that CH_4 oxidizers in the birch site may actually be able to grow on atmospheric CH_4. The surface soils were treated identically to the deeper soils except that they did not experience dramatically increased CH_4 availability upon removal from the field. Hence, the lack of an increase in CH_4 oxidation in the surface soils is also consistent with the hypothesis that the increased CH_4 oxidation in the deeper soils resulted from microbial growth, or at least enzyme synthesis, rather than a disturbance artifact. Similarly, the lack of increased oxidation rates in the spruce soil at any depth also challenges the notion of a disturbance artifact.

The presence of NH_4^+ prevented this physiological upshift, yet it did not inhibit CH_4 oxidation relative to the initial rates on day 1 (Fig. 14c). Thus, although NH_4^+ did not inhibit extant CH_4 oxidation by the organisms in this soil, it repressed their ability to increase their CH_4 oxidation capacity. As discussed previously, this phenomenon may represent inhibition of population growth or enzyme synthesis. Ultimately, the inability to synthesize CH_4–oxidizing enzymes would lead to growth inhibition. From an ecological perspective, therefore, the distinction between growth inhibition and enzyme synthesis inhibition is trivial. This sort of interaction between excess NH_4^+ and methane oxidizer population dynamics is consistent with the delayed inhibition observed in the field: if

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methanotrophs in fertilized plots failed to grow, natural mortality would eventually decrease population size and, in turn, CH_4 consumption rates. The dramatic change from the 1st year of fertilization (no inhibition) to the 2nd year (49% inhibition) is also consistent with this hypothesis. The frozen soils (–6.4°C at 5 cm soil depth in 1992; S.M. Wagener, pers. comm.) and deep snow packs (~1 m) in winter could kill a large proportion of the CH_4–oxidizer community, which would then be unable to recover in fertilized plots.

A physiological mechanism by which NH_4^+ might inhibit growth without inhibiting extant enzyme activity is unclear. One possible mechanism may relate to the energetic cost of oxidizing NH_4^+. The Methane monooxygenase (MMO) enzymes of all known methanotrophs require reducing equivalents to function (Dalton & Higgins 1987). It is unclear whether methanotrophs are able to acquire new reducing equivalents from NH_4^+ (Higgins et al. 1981), but many cultured methanotrophs apparently cannot (Bédard & Knowles 1989). If the CH_4 oxidizers in the birch soil cannot, then growth may be inhibited by diversion of energy from cellular processes required for growth. It is possible for an enzyme to catalyze the conversion of two substrates with negligible competitive inhibition if the total substrate availability remains far below an integrated half–saturation constant (Km) for both substrates (Button et al. 1981; D.K. Button, pers. comm.). Ambient CH_4 supply to well–drained soils is far below estimated Km values for CH_4 consumption in such soils (Whalen et al. 1990; Bender & Conrad 1993; Dunfield & Knowles 1995; Whalen & Reeburgh, in review). Furthermore, the Km of MMO for NH_4^+ in cultured methanotrophs is orders of magnitude higher than for CH_4 (Dalton 1977). It is plausible, therefore, that methanotrophic bacteria in the birch soil oxidize NH_4^+ without competitive inhibition of atmospheric CH_4 oxidation, and that NH_4^+ oxidation places an energetic burden on the population. In a strongly energy–limited environment, even a small energy burden could be sufficient to inhibit growth. Although previous studies have been unable to demonstrate CH_4

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oxidizer growth on atmospheric CH_4 (Steudler et al. 1989; Bender & Conrad 1992; King & Schnell 1994b; Kightley et al. 1995), it has been deemed feasible (Conrad 1984; Bender & Conrad 1993). Regardless of the actual physiological mechanism, however, growth inhibition is consistent with all aspects of our data from the birch site, accounting for both the severe long–term field inhibition, as well as the lack of short–term inhibition in laboratory and field experiments.

Methane Oxidizer Distribution

There is evidence that the methane oxidizer populations in the birch and spruce sites are physiologically distinct (Table 4), which may explain their different responses to fertilization. Unlike the birch soil, the spruce soil exhibited general salt or ion sensitivity, such that sulfates of both K^+ and NH_4^+ inhibited CH_4 oxidation equally (Fig. 13b). This different response to salt occurred despite the fact that the soils in these two sites are physically similar (Viereck et al. 1986; Van Cleve et al. 1991) and exhibit similar osmotic potentials when amended with salts (Chapter IV). Using salt concentrations similar to those in the present study, Kightley et al. (1995) also were unable to distinguish the inhibitory effect of NH_4^+ from those of other ions in microcosms of a landfill cover soil. Another factor distinguishing the birch and spruce sites is that there was no increase in oxidation capacity in the deeper spruce soils during the 10–day incubation at atmospheric CH_4 concentration, whereas there was in the deeper birch soil. Finally, CH_4 oxidation capacity at atmospheric CH_4 concentration in the spruce soil was up to 10–fold higher than in the birch soil, even though field consumption rates were similar (2–year average (± S.E.) for birch: 0.58 ± 0.038 $mg \cdot m^{-2} \cdot h^{-1}$ compared with spruce: 0.52 ± 0.038 $mg \cdot m^{-2} \cdot h^{-1}$). This suggests that maximum field flux rates were restricted by CH_4 diffusion into the soil rather than by biological oxidation capacity (Dörr et al. 1993; Striegl 1993). This observation also explains why CH_4 oxidation capacity declined more precipitously with depth in the spruce site than in the birch

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site (Fig. 12a, b). Although large differences in the CH_4 oxidation capacities are difficult to explain, we have observed this relationship consistently across multiple field seasons. Given the physical similarities of the two soils (Viereck et al. 1986; Van Cleve et al. 1991), it is likely that these contrasting biological characteristics are due to physiological differences between the CH_4 oxidizer populations in these two sites.

Conclusions

We have identified three ecologically distinct patterns of CH_4 consumption inhibition due to fertilization that can be explained on the basis of three physiologically distinct CH_4 oxidizer populations. Immediate inhibition must evoke a fundamentally different physiological response to excess NH_4^+ than does delayed inhibition. Whereas immediate inhibition would likely involve enzyme substrate competition or chemical–ion toxicity, delayed inhibition (as in our birch site) requires an alternative explanation. This mechanism may result in the repression of CH_4 oxidizer growth by NH_4^+. Finally, a complete lack of inhibition in soils with no apparent means protecting CH_4 oxidizers from NH_4^+ (as in our spruce site), is most readily explained by the presence NH_4^+–insensitive CH_4 oxidizers. Such organisms may have limited NH_4^+ transport capacity (King & Schnell 1994a). Thus, physiologically distinct CH_4 oxidizer populations seem to best explain the three inhibition patterns. Actual field inhibition dynamics may not always clearly fit one of these three patterns due to mixed CH_4 consumer communities, or other soil properties regulating exposure of CH_4 oxidizers to NH_4^+. For instance, Castro et al. (1995) observed short-term, but initially mild inhibition of CH_4 consumption in a red pine forest within weeks of fertilization, but inhibition developed to more severe levels over several years. This pattern could result either from a gradual increase in available soil NH_4^+ with repeated fertilization, or from differential inhibition of physiologically distinct CH_4 oxidizer populations.

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Given the ubiquity of soil methane oxidation and low global variation in consumption rates (Bartlett & Harris 1993), it seemed reasonable at first to assume that upland CH_4 oxidizer populations vary little in specific composition and, hence, physiological diversity (Schimel et al. 1993). Mechanistic hypotheses to explain NH_4^+–inhibition dynamics have therefore focused either on seemingly more distal controls, such as soil N dynamics (Mosier et al. 1991; King 1992; Hütsch et al. 1993; Schimel et al. 1993) or ostensibly universal physiologies, such as enzyme substrate competition or NO_2^- toxicity (Bédard & Knowles 1989; Steudler et al. 1989; Carlsen et al. 1991; Schimel et al. 1993; King & Schnell 1994a,b; Schnell & King 1994). Our findings suggest, however, that the primary ecosystem–level control over NH_4^+–inhibition of soil CH_4 consumption may be the distribution of physiologically distinct CH_4 oxidizer populations across sites. Hence, efforts to integrate the effects of fertilization and N deposition into models predicting global CH_4 dynamics may require incorporating regional patterns of CH_4 oxidizer distribution. At the landscape scale, NH_4^+–inhibition appears too complex to describe using a single mathematical relationship.

Future Directions

Our findings show, in agreement with previous studies (Nesbit & Breitenbeck 1992; Adamsen & King 1993; Bender & Conrad 1994; Crill et al. 1994; Schnell & King 1994), that NH_4^+–inhibition of soil CH_4 oxidation is a complex and enigmatic process that is difficult to assign any single cause. This work raises several key questions regarding the ecology of soil methane oxidizers. First, if the occurrence of physiologically distinct CH_4 oxidizers determines NH_4^+–inhibition dynamics in different ecosystems, what controls the distribution of the different CH_4 oxidizer populations? Is it related to N cycling dynamics or some other aspect of the ecosystem? Are distinct populations merely different strains of methanotrophs, or fundamentally different organisms (*e.g.*, nitrifiers or unknown

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mixotrophs)? Methanotrophs growing on atmospheric CH_4 would probably be oligotrophic, and may have escaped isolation due to the use of enrichment culture techniques, which select for more copiotrophic organisms (Bender & Conrad 1993).

This work provides a set of testable hypotheses capable of explaining all inhibition dynamics observed in the present study, and also the variety of seemingly inconsistent results that have been published regarding NH_4^+–inhibition. Clearly, more work is needed to determine the actual physiological mechanism(s) of inhibition in our upland taiga soils, and whether there are methanotrophs that actually grow solely on atmospheric CH_4. To date, the organisms responsible for atmospheric CH_4 oxidation have eluded isolation, but comparative studies of isolates from ecosystems exhibiting various responses to N fertilization would be useful in answering many of these questions.

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Table 4. Distinct physiological responses of CH_4 oxidizers to NH_4^+ additions in two soils incubated with atmospheric CH_4 (1.8 ppm)

site	inhibition pattern	salt sensitivity	apparent growth on 1.8 ppm CH_4	maximum oxidation rate (pmol•g $soil^{-1}$•h^{-1})
paper birch	delayed	no	yes	7.9
white spruce	none	yes	no	76.0

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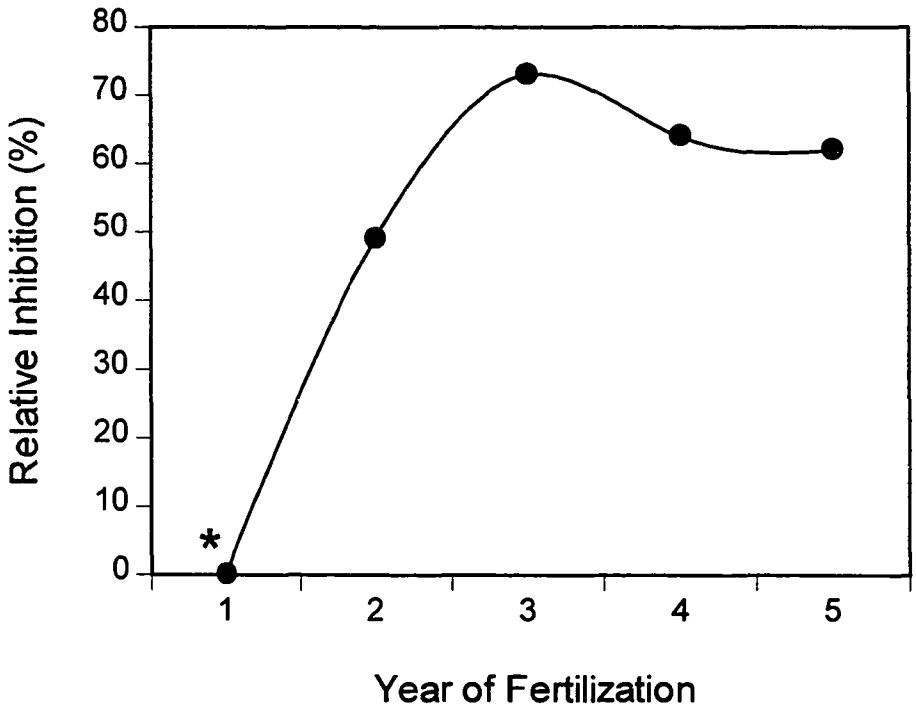

Figure 11. Inhibition of soil CH_4 consumption by NH_4^+–fertilization in a birch site, expressed as per cent of the control. Data for year 1 (*) are from the secondary plots established in 1993. The remaining data are from the primary plots established in 1990. Points represent the average of data collected every two weeks throughout each snow–free season.

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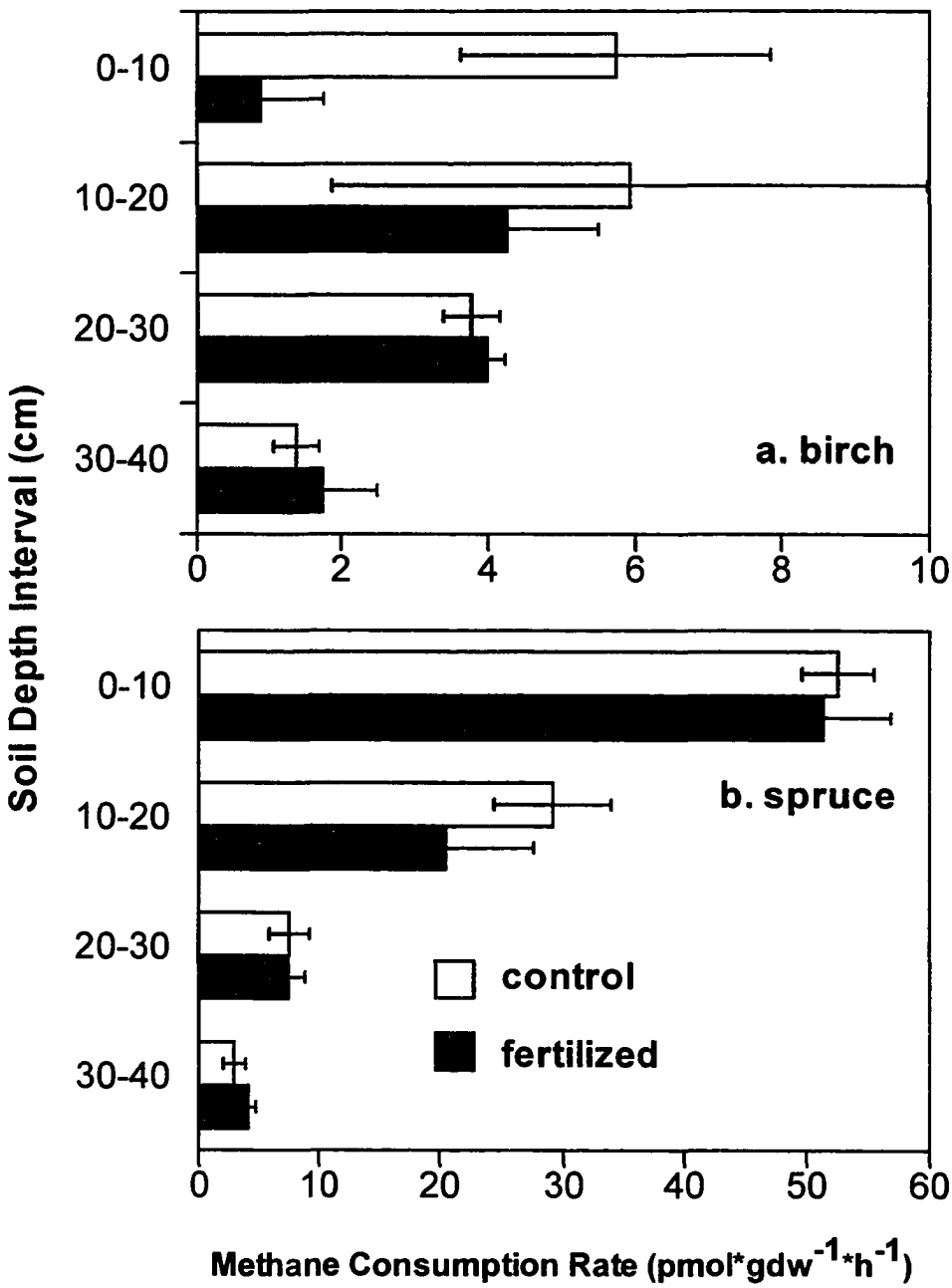

Figure 12. Depth profile of CH_4 oxidation capacity in fertilized and control soils from the birch (a) and spruce (b) sites. The depth scale represents mineral soil depth rather than depth from the forest floor surface. Soils were incubated with atmospheric CH_4. Error bars = ±SE.

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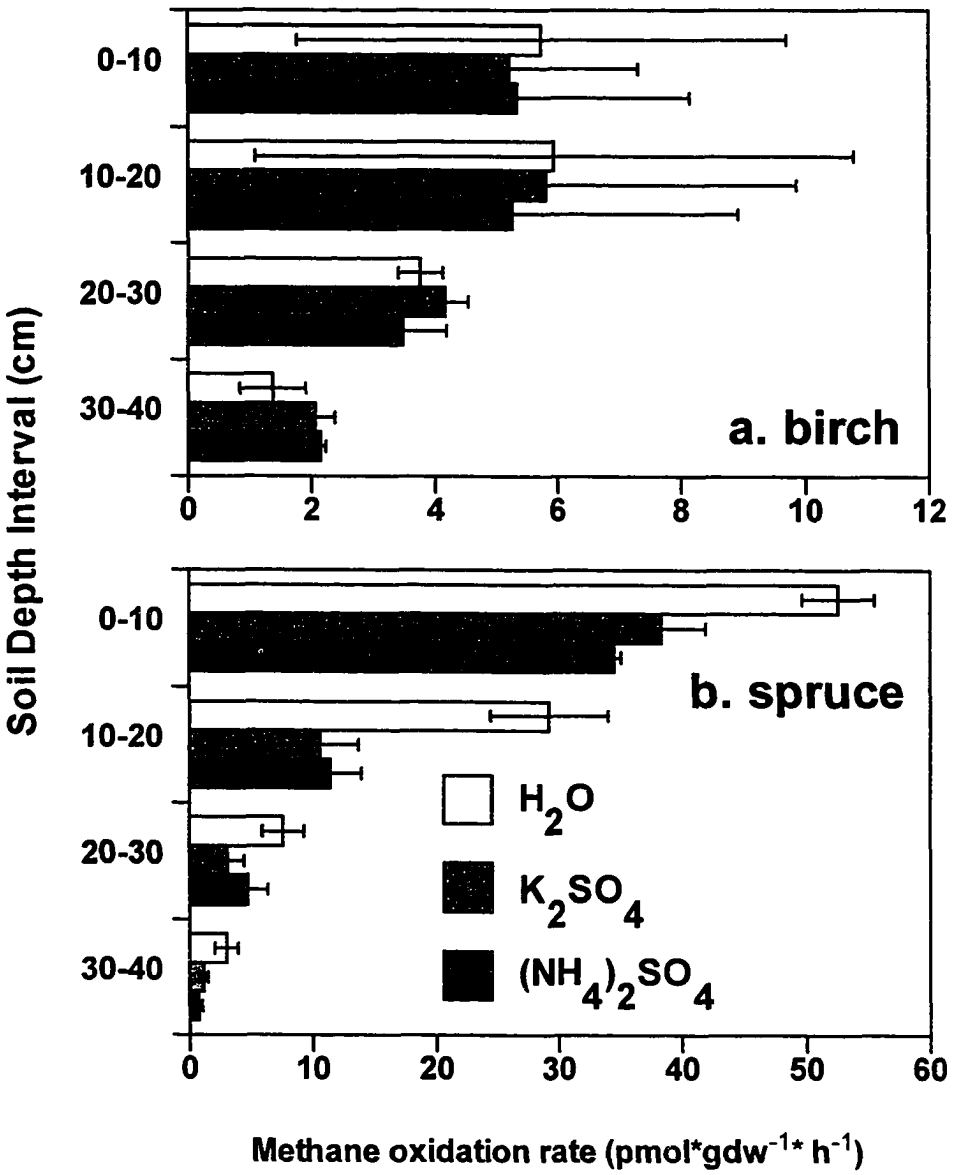

Figure 13. Effects of K_2SO_4 and $(NH_4)_2SO_4$ additions on CH_4 oxidation capacity in control soils from birch (a) and spruce (b) sites. Salt solutions were added at a rate of 2.7 µmol•(g dry soil)$^{-1}$. The depth scale represents mineral soil depth rather than depth from the forest floor surface. Soils were incubated with atmospheric CH_4. Error bars = ±SE.

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Figure 14. CH_4 oxidation capacity in control birch soil on the first (Day 1) and tenth (Day 10) day of incubation with H_2O (a) K_2SO_4 (b) and $(NH_4)_2SO_4$ (c) added. The depth scale represents mineral soil depth rather than depth from the forest floor surface. Soils were incubated with atmospheric CH_4. Error bars = ±SE.

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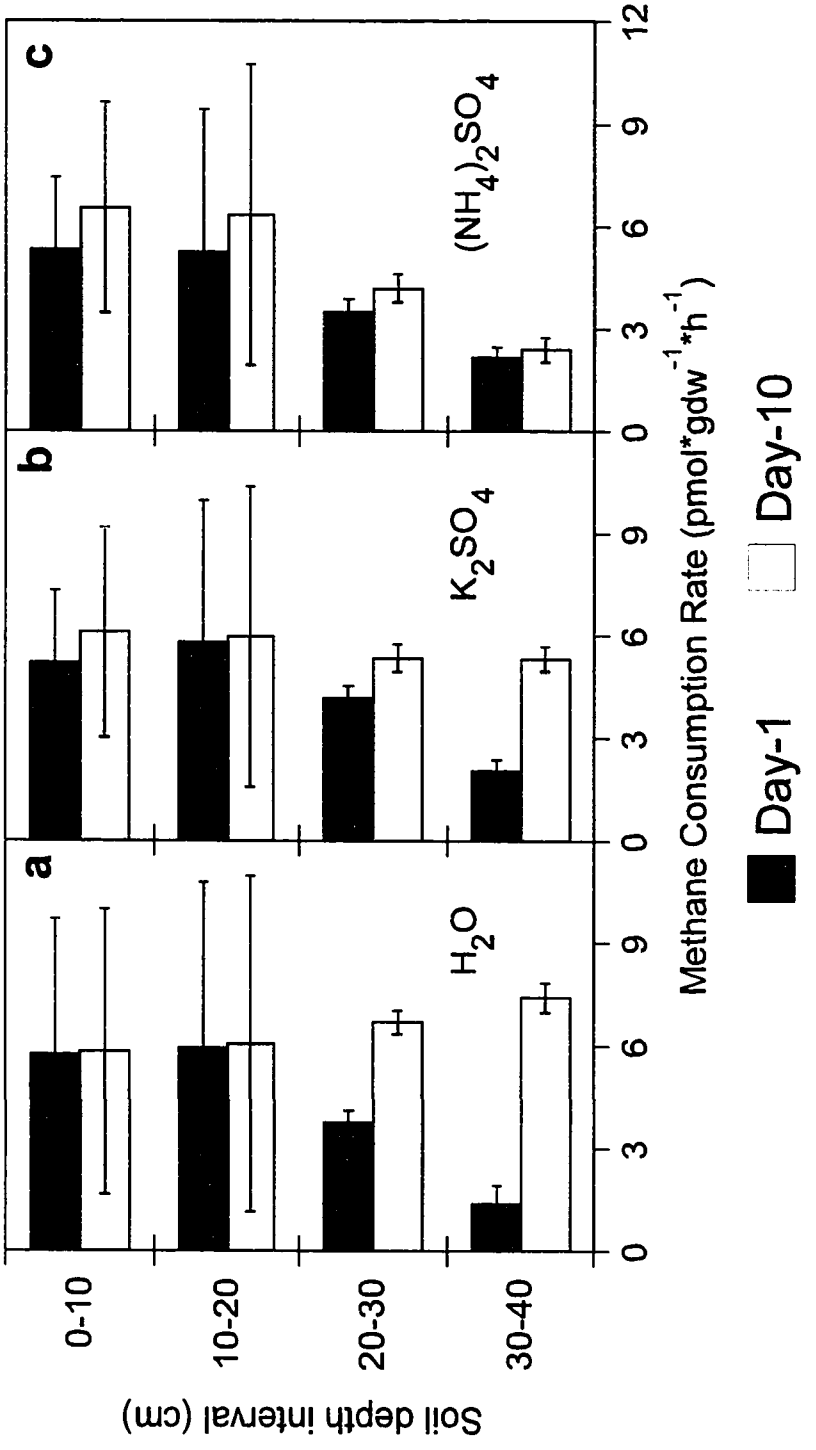

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Figure 15. Net N mineralization in birch and spruce soil without NH_4^+ added (a) and with 75 µg NH_4^+–N added (b). The depth scale represents mineral soil depth rather than depth from the forest floor surface. Error bars = ±SE.

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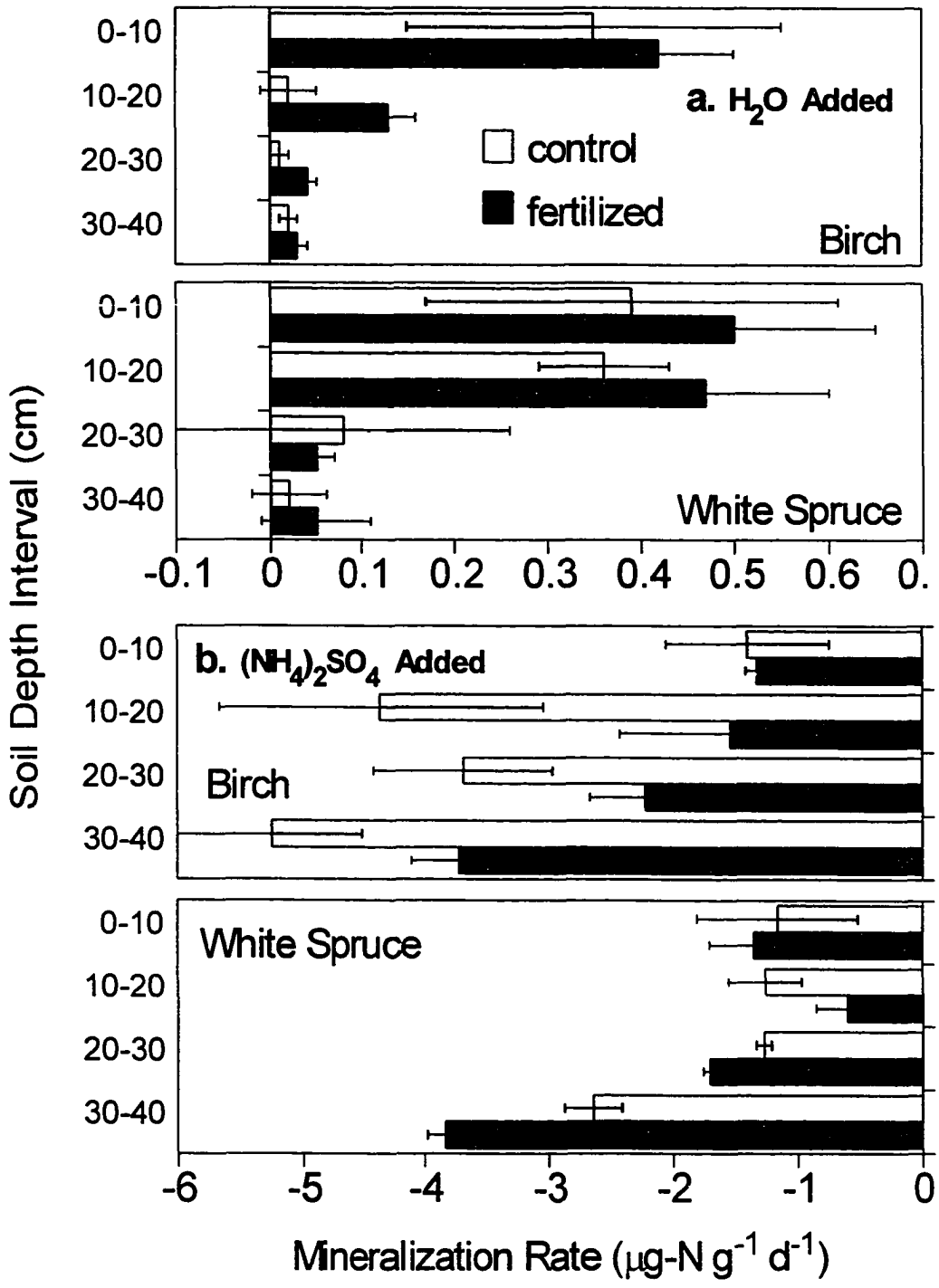

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VI. Synopsis & Conclusions

With this project I sought to address our general lack of knowledge regarding the ecosystem–level ecology of CH_4 consumption in soils and the ecophysiology of the organisms involved. Until recently, essentially no information was available regarding the process ecology of soil CH_4 consumption. Steudler et al. (1989), working in temperate forests, were the first to report NH_4^+–inhibition of CH_4 consumption. Shortly thereafter, similar results were reported in tropical forests (Keller et al. 1990), and temperate grasslands (Mosier et al. 1991). Together, these early field–based studies brought to light the extreme sensitivity of the process to disturbance in vastly different ecosystems. This sensitivity has been reconfirmed time and again in a diverse array of ecosystems (Chapter V). Only within the time frame of this project have researchers turned their attention to the physiological mechanisms by which disturbance (usually meaning NH_4^+–fertilization) inhibits CH_4 oxidation. These efforts have been hampered, however, by the fact that the responsible organisms have not been isolated or identified. Hence, those of us studying the biological basis of inhibition have had to infer from process studies the physiological nature of the organisms. This is a difficult task, requiring some degree of speculation. My approach was to reduce the degree of speculation required by learning more about the organisms.

The Organisms

With this philosophy in mind, the CH_4–starvation experiment described in Chapter II was designed to determine whether the CH_4 oxidizers in upland taiga soils intentionally oxidize atmospheric CH_4, or whether this activity is merely fortuitous, resulting from incidental binding of CH_4 to one or more enzymes intended to oxidize other substrates. In both soils examined, CH_4 served to

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maintain the enzymes that oxidized it. I cannot say whether the organisms were dying (or becoming dormant) due to CH_4 starvation, or whether the CH_4–oxidizing enzyme system was simply deactivated. Nonetheless, the results clearly indicate that the organisms target and metabolize atmospheric CH_4. This is an important conclusion because it was not obvious that this should be the case. Methanotrophs known from pure cultures cannot survive on atmospheric CH_4 because its concentration is too low to sustain them (Conrad 1984). Furthermore, despite the fact that the bacteriological literature is replete with reports of facultative methanotrophs (bacteria that utilize CH_4 in addition to other C substrates), no methanotrophic cultures confirmed to be pure are known to acquire C from sources other than CH_4 (Lidstrom 1992).

I should point out that it is hardly safe to assume that these organisms survive *solely* on atmospheric CH_4 based on this experiment, alone. Deactivation of CH_4 oxidation does not prove that the organisms require atmospheric CH_4 for survival, it simply indicates that they benefit from it. Given what is known about CH_4–oxidizing bacteria, however, the most acceptable conclusion from this study is that the soil organisms that oxidize atmospheric CH_4, at least in nonnitrifying soils, are methanotrophs. Fortunately, the experiment described in Chapter V supports this conclusion, since it suggests that the CH_4 oxidizers in the birch forest soil are able to grow on atmospheric CH_4. Schnell & King (1995) found that a plethora of non–CH_4 C sources failed to support growth of atmospheric CH_4 oxidizers. Thus, they may rely solely on atmospheric CH_4. If so, they are, by definition, obligate methanotrophs. It is interesting that there may be a ubiquitous group of oligotrophic, obligate methanotrophs that is distinct from those more copiotrophic species that have been studied in pure culture for nearly a century.

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Controls on Soil CH_4 Consumption

Controls on soil CH_4 consumption may be distal, which I define as those factors that control the supply of needed substrates (CH_4, O_2) to the organisms, or proximal, which are those factors that act directly on the organisms, affecting their ability to oxidize CH_4, even when the required substrates are available. Moisture may be either a distal or a proximal control. It is perhaps the most important distal control, since it directly regulates CH_4 and O_2 availability in many soils. As a proximal control, low moisture reduces the activity of soil microbes in general, including CH_4 oxidizers, due to water stress. The most extensively studied proximal control on soil CH_4 oxidation has been NH_4^+–inhibition. Despite the recent flurry of studies on this phenomenon, it has been difficult to determine its mechanism, and thus, to predict its dynamics in the field. Changes in soil moisture and N–fertilization–deposition may represent two of the most important disturbances affecting the soil CH_4 sink, because they accompany a large proportion of land–use changes. My goal was to understand how these two factors affect not just the process of soil CH_4 consumption, but the CH_4 oxidizers themselves.

NH_4^+–Inhibition

Studies of NH_4^+–inhibition require chemical alteration of soil conditions by increasing the concentrations of dissolved ions. Anytime soil chemistry is manipulated, care must be taken to consider the physiological effects of these changes on the organisms under examination. NH_4^+ is not the only ion added to the soil, since it must be accompanied by an anion, and the potential effect of this anion must be considered, as well. Furthermore, NH_4^+ may have more than one mode of inhibition. It may be specifically inhibitory, or it may exert a nonspecific effect that similar cations also would impose. As demonstrated in Chapter IV, it is crucial to control for these effects. Many studies of NH_4^+– inhibition have not done so, and may have therefore interpreted the toxicities of Cl^- or other non–NH_4^+ ions

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as an NH_4^+ effect. My results suggest that the high degree of NH_4^+–inhibition frequently reported in the literature often is due to what may be deemed salt effects, rather than NH_4^+, *per se*. As a result, the global magnitude of NH_4^+ as an inhibitor of soil CH_4 consumption may have been over–estimated. This is not to say that fertilization effects are not important, simply that NH_4^+ is only one factor involved in the total amount of inhibition observed.

Many researchers have sought a single physiological inhibition mechanism for NH_4^+. Given the ubiquity of soil methane oxidation and low global variation in consumption rates, it seemed reasonable at first to assume that upland CH_4 oxidizer populations vary little in specific composition and, hence, physiological diversity. Mechanistic hypotheses to explain NH_4^+–inhibition dynamics have therefore focused either on seemingly more distal controls, such as soil N dynamics or ostensibly universal physiologies, such as enzyme substrate competition or NO_2^- toxicity. Our findings suggest, however, that the primary ecosystem–level control over NH_4^+–inhibition of soil CH_4 consumption may be the distribution of physiologically distinct CH_4 oxidizer populations across sites. Diversity among these organisms may explain why inhibition dynamics vary.

Organismal diversity also suggests that inhibition mechanisms may vary. A strong argument can be made from several studies that substrate competition (between CH_4 and NH_4^+) is often involved. Another possibility is inhibition of growth, followed by diminished populations, as described in Chapter V, where NH_4^+ additions appeared to halt growth of the methanotroph population in the upland birch soil. Toxicity of NO_2^- or NH_2OH produced by fortuitous NH_3 oxidation is a third possibility, although the evidence for this mechanism is dubious due to the lack of control for nonspecific salt effects in the studies from which this hypothesis emerged (Chapter IV).

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Soil moisture

The study described in Chapter III demonstrated that CH_4 oxidation exhibits a parabolic response to soil moisture, with an optimum occurring at the moisture content that allows the maximum rate of CH_4 supply to the organisms, while minimizing water stress. This result is neither surprising, nor novel; the significance of this work lies elsewhere. First, it demonstrates that the effect of soil moisture on CH_4 oxidation is quantitatively similar among soils of vastly different physical structure, providing that soil moisture is expressed relative to the water holding capacity of the soil. This result is important because quantifying soil moisture in this fashion would facilitate communication and comparison of results across sites, and may be useful in dynamic modeling of ecosystem CH_4 consumption. Most researchers have expressed soil moisture in absolute terms, which makes cross–site comparison meaningless unless the soils are structurally similar, and would require significant re–parameterization of CH_4 flux models when shifting from one soil type to another. Second, this work is the first (as far as I am aware) to examine the effect of water potential on CH_4 oxidation in soil. One interesting result is that water potential had a similar relationship with %WHC in soils of vastly different physical and chemical composition (Fig 6, Chapter III). Also, maximum CH_4 oxidation occurs at a water potential consider-ably lower than that favoring general microbial acitivity (Fig. 4, Chapter III).

The data suggest that CH_4 consumption in the upland taiga soils of interior Alaska may be sensitive to climate change. In my experience, field moisture in these soils usually hovers between 20–40% of water holding capacity, which is the range of optimum moisture for CH_4 consumption. Either wetter or drier climate conditions could therefore diminish the CH_4 sink in these soils. Conversely, peat soils, such as the wetland taiga soil, would be relatively insensitive to drying. Thus, organic–rich soils may be poised to provide a strong atmospheric CH_4 sink should dryer conditions prevail. This conclusion agrees with that of Whalen and Reeburgh

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(1990) that moist tundra soils would become a net CH_4 sink under warmer, dryer conditions in the Arctic.

Final Conclusions & Future Directions

From this body of work, and the data available from published studies, I have arrived at the following conclusions regarding the ecology of the soil CH_4 sink. Although I have chosen to state these conclusions as fact, the reader should be aware that they represent what I believe are the most reasonable hypotheses based upon available data. They are listed in order of importance (most important first) from my perspective. The methodological conclusions, although critical, are relegated to the end of the list in favor of the more conceptual conclusions, which I deem to be the more substantial contributions to our understanding of the soil CH_4 sink.

1. Soil CH_4 oxidizers are essentially methanotrophic, but physiologically diverse. Thus, NH_4^+–inhibition dynamics and mechanisms vary among sites, and some soils are insensitive to NH_4^+.
2. Some soil methanotrophs can grow on atmospheric CH_4, though this ability varies among ecosystems according to the CH_4 geochemistry of the site.
3. In some ecosystems, NH_4^+ diminishes the soil CH_4 sink by inhibiting methanotroph growth, rather than extant CH_4 oxidation.
4. Controlling for salt effects when conducting NH_4^+–inhibition studies is crucial. Lack of such control in a number of published studies has resulted in over-estimation of the significance of NH_4^+–inhibition of soil CH_4 consumption.
5. NO_2^-–inhibition of methanotroph activity is a proposed mechanisms that has received much attention recently. Because of inappropriate experimental design, however, it is likely that the inhibition dynamics from which this hypothesis was inferred were due to Cl^- rather than NH_4^+.

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6. Optimum water content (35% WHC) is consistent among sites with different soil properties. Percent WHC is a useful expression of soil moisture because it normalizes water content against water filled pore space and water potential in soils with widely differing physical properties.

I believe these conclusions significantly advance our understanding of the biological nature of soil CH_4 consumption and the organisms involved. Only in the past couple of years has our understanding of the physiologi-cal ecology of soil CH_4 oxidation made significant strides, and much remains to be learned. I believe future research should focus on three general areas:

1. the diversity and mechanisms of factors other than NH_4^+ that inhibit soil CH_4 oxidation;
2. isolation, characterization, and diversity assessment of soil CH_4 oxidizers in various ecosystems;
3. development of molecular techniques and other tools for studying the distribution and ecology of atmospheric CH_4 oxidizers *in situ*.

Naturally, these areas should accompany continued work on the basic biogeochemistry of soil CH_4 cycling. I also believe it is important for microbial ecologists to join efforts to model ecosystem CH_4 flux, in order to incorporate biological information into dynamic models.

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